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Ionization of Sulfonamides.

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A recent quantitative analysis of bacteriostasis by sulfanilamide, sulfapyridine, sulfadiazine and sulfathiazole showed that for inhibition of *Es. coli* decreasing amounts of each drug in the order named were required.[1] Actually over six hundred times more sulfanilamide is required than sulfathiazole or sulfadiazine, and similar results have also been obtained with a variety of other microörganisms,[2] indicating that this regular increasing order of bacteriostatic potency is characteristic of these N_1 sulfonamides (Col. A, Table I) and not attributable to the selective action of one drug against a certain organism. Furthermore, the quantitative relationships between these sulfonamides and para aminobenzoic acid (PAB) differ just as widely; *e. g.*, over 600 times more PAB is needed to block sulfathiazole and sulfadiazine than to block sulfanilamide. (Col. F.) When the minimum effective concentration of each drug was tested, the same amount of PAB was required to block each drug. (Col. E.) In other words, going from sulfanilamide to sulfadiazine a progressively larger proportion of each drug actually present in the cultures appears to possess activity against either bacteria or PAB; the active moiety presumably being similar for each drug.

It seems possible that such a partition into active and inactive portions might be controlled by the extent to which the drugs are ionized in the cultures. Accordingly the dissociation constants were measured* (Col. B) and the percent of each drug ionized at pH 7.0 (Col. C) was computed. (Some shift in the apparent pK may be expected to occur in complex biological media.) Based on these values and using the minimum effective amounts of drug added to the culture (Col. A), the concentration of ionized drug was estimated

1 Rose, H. M., and Fox, C. L., Jr., *Science*, 1942, **95**, 412.

2 Fox, C. L., Jr., and Rose, H. M., unpublished data.

* The drugs were dissolved in CO_2 free water and titrated with 0.01 N NaOH using the Beckman glass electrode and potentiometer. The pK_A values were also obtained by measuring the pH of equimolar mixtures of the drugs and their sodium salts in CO_2 free water. The drug concentrations used were about 0.001 M.

TABLE I.
Ionization of Sulfonamides and Effective Concentrations of the Drugs.

	(A) Min. effective drug conc., M × 10⁻⁶	(B) Acid dissociation constant, pK_A	(C) % ionized at pH 7.0	(D) Conc. of ionized drug pH 7.0 M × 10⁻⁶	(E) Min. amt PAB required to prevent bacteriostatis with drugs, Col. A, M × 10⁻⁶	(F) PAB / Drug Ratio	(G) PAB / Ionized drug (Col. D)
Sulfanilamide	2500	10.5	0.03	0.71	0.5	1-5000	1/1.4
Sulfapyridine	20	8.5	3.4	0.68	0.5	1-40	1/1.4
Sulfathiazole	4	6.8	61.6	2.46	0.5	1-8	1/4.9
Sulfadiazine	4	6.4	80.0	3.2	0.5	1-8	1/6.4

The pK_A of para aminobenzoic acid is 4.9.[8,9] Its influence on the ionization of these drugs is under investigation.

(Col. D). From these values, the ratios PAB/Ionized Drug were obtained (Col. G).

A comparison of Cols. B and A shows that the variations in the dissociation constants and in the minimum effective concentrations are in the same order. More significant, however, is the fact that the concentration of ionized drug is approximately the same in cultures containing very different amounts of these drugs. Comparison of Cols. F and G shows that the wide differences in the PAB/Drug ratios are sharply narrowed and the ratios PAB/Ionized Drug are of the same order of magnitude.

From these data certain important inferences may be drawn. It appears that only the ionized portions of the sulfonamide acids exert antibacterial action. Furthermore, the minimal amount of each drug (ionized portion) needed to obtain bacteriostasis is approximately the same. In other words it is possible that the relative bacteriostatic potency of these drugs is only a measure of their ionization at biological hydrogen ion concentrations. To test this inference the minimal bacteriostatic concentration of sulfanilamide was measured at pH 6.8 and at pH 7.8. Although growth in the controls was the same at both pH levels, 20,000 μM per L of drug were required for bacteriostasis at pH 6.8, whereas only 2,500 μM per L were needed at pH 7.8. Since the ionization of sulfanilamide at pH 7.8 is increased ten-fold over that at pH 6.8, the amounts of ionized drug in the cultures are actually similar, despite the widely differing total amounts of drug employed. The significance of these observations in terms of a mechanism of sulfonamide action needs further study. Woods postulated competition for a bacterial

[8] Holmberg, *Z. f. Phys. Chem.*, 1908, **62**, 728, cited in Beilstein, 1931, **14**, 419.
[9] Albert, A., and Goldacre, R., *Nature*, 1942, **149**, 245.

enzyme between one mol of an essential metabolite, PAB, and 5-25,000 mols of sulfanilamide.[3] It is apparent from the previous experiments[1] that the quantity of enzyme (number of bacterial cells) is irrelevant. The present data suggest that all the drugs react in roughly similar amounts with approximately equivalent quantities of PAB.

There are, in addition, important physiological implications. Since at the pH of body fluids sulfanilamide on the one hand is almost completely unionized and sulfadiazine is almost completely ionized, the question might well be raised as to the relative effectiveness of similar blood levels of these drugs. There is a possibility that in therapy lower blood levels of sulfathiazole and sulfadiazine might be just as effective since concentrations in excess of the minimum effective level do not further retard bacterial growth.

This difference in degree of ionization also exercises considerable control over the solubility of these drugs in biological fluids. Table II shows that the low water solubility of sulfadiazine is increased 14 fold in M/15 phosphate buffer at pH 7.4. It is apparent that these data may account to a large extent for the differences in the absorption, distribution and excretion of these drugs. More detailed data on these points together with related data on the acetylated derivatives will be published elsewhere.[4] It also seems possible, however, that these data explain Marshall's observation[5] that sulfadiazine (ionized) seems confined to extracellular fluid whereas sulfanilamide (unionized) is distributed with body water.[6] Finally, utilization of

TABLE II.
Influence of Ionization on the Solubility of Sulfonamides.

	Solubility in H_2O 37°C	Solubility in M/15* phosphate buffer pH 7.4	No. of times increase in solubility
Sulfanilamide	1,400 mg per 100 cc	1,720 mg per 100 cc	1.2
Sulfapyridine	49.5 "	75 "	1.5
Sulfathiazole	94 "	248 "	2.6
Sulfadiazine	12.3 "	167 "	13.6

*All measurements were made after filtering off the excess solid phase of drug at 37°C. Drug concentrations were estimated by modifications of the method of Bratton and Marshall[10] using the Klett-Summerson photo-electric colorimeter. The pH of approximately 7.4 was obtained after the drugs were dissolved in buffers of higher pH.

3 Woods, D. D., *Brit. J. Exp. Path.*, 1940, **21**, 74.

4 Jensen, O., and Fox, C. L., Jr., in preparation.

5 Marshall, E. K., Jr., Conference on Chemotherapy, the New York State Department of Health, 1941.

6 Painter, E., *Am. J. Phys.*, 1940, **129**, 744.

10 Bratton, A. C., and Marshall, E. K., Jr., *J. Biol. Chem.*, 1939, **128**, 537.

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.