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Studies on Antibacterial Action of Sulfonamide Drugs. III. Correlation of Drug Activity with Binding to Plasma Proteins.

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In a preliminary report[1] a correlation was noted between the bacteriostatic power and the protein-binding tendency of 4 sulfonamide drugs: sulfanilamide, sulfapyridine, sulfadiazine, and sulfathiazole. It has been suggested by Woods[2] that sulfonamide bacteriostasis depends upon specific inhibition of an enzymatic reaction involving p-aminobenzoic acid. Further evidence has recently been presented[3-5] supporting this concept. Since all known enzymes are proteins, and since the inhibition of an enzymatic reaction may involve some form of chemical interaction between inhibitor and enzyme, it seemed worthwhile to investigate further the interaction between sulfonamide drugs and proteins. The present paper reports data on the binding to plasma proteins of a larger series of sulfonamide drugs and related compounds. The significance of the data is briefly discussed.

Methods. The method of determining the binding of drug to plasma protein, which is described in detail elsewhere,[6] consisted essentially of determining the concentration of drug in samples of plasma and buffer (pH 7.4) which had been dialyzed against each other until equilibrium was reached. The concentration of drug in the plasma in excess of that found in the buffer was considered to be bound to the protein; the data are reported as the percentage of the value of the unbound drug which is bound per g of protein. All the experiments were performed with a single lot of plasma.

The method of determining the bacteriostatic activity of the various sulfonamide drugs has been described in a previous paper.[4] The minimum concentration of drug, which would prevent visible growth of a controlled inoculum of *E. coli* incubated in a synthetic medium for 5 days at 37°C, was arbitrarily selected as the bacteriostatic end point.

Results. Binding of p-Amino Benzene Sulfonamides. In Table I are presented data on the binding to plasma of 7 homologues of the p-amino series which have in common the p-amino radical but vary in the groups attached to the sulfonamide portion of the molecule. The minimum bacteriostatic concentrations of each drug against *E. coli* are also recorded in Table I. Although the measurement of relative bacteriostatic activity is admittedly crude, there is demonstrated a close correlation between binding tendency and bacteriostatic potency. While this agreement may be coincidental, it suggests that the tendency of a given drug to be bound to plasma protein depends upon properties which likewise are of importance in the mechanism of bacteriostasis.

Binding of Related Compounds. Sulfonamide compounds which do not contain the p-amino group also become bound to protein. It is seen from the data in Table II that ortho- and meta-amino benzene sulfonamide, the less active isomers of sulfanilamide, show as much, and in the case of orthonilamide even more binding than sulfanilamide. Also the acetyl derivatives of sulfanilamide, sulfa-

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1 Davis, B. D., *Science*, 1942, **95**, 78.

2 Woods, D. D., *Brit. J. Exp. Pathol.*, 1940, **21**, 74.

3 Wyss, O., Grubaugh, K. K., and Schmelkes, F. C., PROC. SOC. EXP. BIOL. AND MED., 1942, **49**, 618.

4 Wood, W. B., Jr., *J. Exp. Med.*, 1942, **75**, 369.

5 Wood, W. B., Jr., and Austrian, R., *J. Exp. Med.*, 1942, **75**, 383.

6 Davis, B. D., in press.

TABLE I.
Binding of Sulfonamide Drugs to Plasma at pH 7.4.

Drug	Con. (M. × 10-4) of drug in buffer	Con. (M. × 10-4) of drug in plasma	Conc. protein (g%)	% of free drug bound per g protein	Avg min. bacteriostatic conc. (M. × 10-5)
Sulfanilamide	1.01	1.16	5.96	3.2	40
	1.85	2.13	5.38	3.5	
	1.84	2.12	5.63	3.3	
Sulfacetamide	0.98	1.19	5.45	4.6	20
	1.86	2.16	5.68	3.5	
	1.84	2.13	5.70	3.3	
Sulfaguanidine	0.60	0.75	5.90	5.1	20
	1.75	2.15	5.22	5.2	
	1.73	2.08	5.56	4.3	
Sulfapyridine	0.55	0.80	5.07	9.7	2
	1.5	2.5	5.21	13.6	
	4.7	6.9	5.16	10.0	
	10.0	14.4	5.18	9.3	
	19.6	26.0	4.89	8.2	
Sulfapyrazine	0.48	1.08	5.94	22.0	2
	1.5	2.7	6.22	14.0	
	1.55	3.40	5.21	24.0	
	3.0	5.1	5.50	14.0	
Sulfadiazine	0.50	1.03	5.11	22.0	2
	1.75	2.95	5.38	14.0	
	2.9	4.8	5.07	14.0	
	8.9	13.2	5.59	9.5	
	20.4	31.8	4.71	13.0	
Sulfathiazole	0.48	1.58	5.31	44.0	0.8
	1.50	4.6	5.45	39.0	
	4.4	10.6	5.72	25.0	
	8.7	18.0	4.76	23.0	
	18.0	34.2	5.44	17.0	

pyridine, sulfadiazine, and sulfathiazole, which are without bacteriostatic activity, are nevertheless bound to approximately the same degree as the corresponding unacetylated drugs.

Discussion. In the case of 7 of the most widely employed sulfonamide drugs there has been demonstrated a close correlation between bacteriostatic potency and the tendency to bind plasma protein. That the bacteriostatic activity of each drug is not determined by its power to bind protein is indicated by the fact that bacteriostatically inactive sulfonamide derivatives show the same tendency to bind protein. Data are presented by one of us[6] to support the conclusion that the dissociated anion of the sulfonamide compounds is essentially the form which becomes bound. On this basis the correlation of binding tendency with bacteriostatic potency can be adequately explained, for Schmelkes *et al.*,[7] and Fox and Rose[8] have recently demonstrated a striking parallelism between the acid-base dissociation constants and the bacteriostatic activities of a series of sulfonamide drugs. If only charged sulfonamide ions are bound to protein, the binding tendency of a given drug would be expected to be proportional to its dissociation constant and thus to its bacteriostatic activity. The fact that bacteriostatically inactive sulfonamide derivatives likewise bind protein is not inconsistent with this hypothesis, for there is conclusive evidence that the antibacterial properties of the sulfonamide drugs depend

[7] Schmelkes, F. C., Wyss, O., Marks, N. C., Ludwig, B. J., and Strandskov, F. B., PROC. SOC. EXP. BIOL. AND MED., 1942, **50**, 145.

[8] Fox, C. L., Jr., and Rose, H. M., PROC. SOC. EXP. BIOL. AND MED., 1942, **50**, 142.

TABLE II.
Binding of Related Compounds to Plasma at pH 7.4.

Drug	Conc. (M. × 10-4) of drug: buffer	Conc. (M. × 10-4) of drug: plasma	Conc. protein (g%)	% free drug bound per g protein
P-amino benzoic acid	0.55	0.70	5.71	5.4
	1.60	1.75	6.11	2.3
Sulfanilic acid	0.87	0.96	(Assume	2.5
	1.60	1.75	5.5)	2.3
Orthonilamide	0.55	0.80	5.12	9.6
	1.65	2.50	6.14	9.0
Metanilamide	0.58	0.68	5.44	3.9
	1.75	2.10	5.52	4.4
Acetyl sulfanilamide	5.1	7.0	5.45	7.5
" sulfapyridine	3.4	7.0	5.37	20.0
" sulfadiazine	3.0	5.4	5.62	15.0
" sulfathiazole	1.1	5.0	5.37	65.0

not only upon molecular dissociation but also upon the presence of the proper structural relationship in the dissociated anion.[2]

Summary. The protein-binding power of 7 commonly employed sulfonamide drugs has been measured by dialysis experiments. A quantitative correlation has been demonstrated between the protein-binding tendency and the bacteriostatic activity of the 7 drugs. Alteration of the p-amino structure of the compounds failed to affect significantly the protein-binding power although it destroyed the antibacterial properties. The data presented here and in another publication by one of us support the recently advanced hypothesis that the anionic species of the sulfonamide molecule is the active component involved in the mechanism of bacteriostasis.

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Action of Bacterial Toxins on Tumors. II. Effect of Sulfanilamide on Toxin-Induced Hemorrhage.

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It has been pointed out[1] that the lethal action of *Salmonella* endotoxin is markedly reduced *in vivo* by the administration of various sulfanilamide compounds. This lethal factor parallels in action the hemorrhagic factor present in the endotoxins of most gram-negative bacteria.[2] The parallelism of action in respect to sulfanilamide, as indicated by the following experiments, suggests the possibility that the two factors are similar, if not identical.

The toxic material used in this study was made by growing *Salmonella typhimurium* in a synthetic liquid medium. The methods for extraction have been previously described.[1] A single uniform batch of antigenic material representing the growth from forty liters of medium was used in both this and the previous study.[1] Observations of hemorrhagic effect were made on 7-day-old implanted tumors (Mouse Sarcoma 180).

Rockland mice bearing tumors were given

1 Hutner, S. H., and Zahl, P. A., *Science*, in press.

2 Zahl, P. A., Hutner, S. H., Spitz, S., Sugiura, K., and Cooper, F. S., *Am. J. Hyg.*, 1942, **36**, 224.