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.

TABLE I.
Effect of Orally Administered Sulfanilamide on the Tumor-Hemorrhage Action of *Salmonella* Endotoxin.

Amt of toxin (μg intraperitoneal)	Amt of sulfanilamide (mg oral)	Degree of tumor hemorrhage				Total No. animals
		—	+	++	+++	
1300	0			3	9	12
1300	20			5	11	16
665	0			4	7	11
665	20	1		6	6	13
530	0			2	8	10
530	20			4	9	13
265	0			2	7	9
265	20		1	2	10	13
165	0			2	12	14
165	20		9	2	4	15
100	0		4	3	24	31
100	20	25	7	4	8	44
66	0		2	4	15	21
66	20	16	2	5	6	29
33	0	2	6	4	10	22
33	20	19	6	2		27
12.8	0	2	1	6	1	10
6.4	0	6	2	3	3	14
1.6	0	6	1			7

20 mg of neutralized sulfanilamide by stomach tube, followed by the intraperitoneal administration of the toxin in aqueous solution. Six to 10 hr after treatment the tumors were examined for hemorrhage.

From the table it is seen that the minimum *hemorrhage* dose is about 6.5 μg of dry weight of toxic material.* The minimum hemorrhage dose (M.H.D.) is defined as the smallest quantity of antigen which, when injected intraperitoneally, causes from slight (+) to marked (+++) hemorrhage in the tumors of 50% of the tested animals.

The minimum *lethal* dose of the endotoxin preparation used in this and the previous study[1] was approximately 1.3 mg for non-tumor-bearing mice. When this amount of the material was injected into tumor-bearing mice protected against death by adequate sulfanilamide treatment, marked hemorrhage occurred in the tumor, indicating that sulfanilamide does not negate the hemorrhage factor of the antigen at this dosage of the antigen. It is seen in the table that as the dosage of endotoxin was reduced respectively to 665, 530 and 265 μg, sulfanilamide remained ineffective so far as obviating the hemorrhage. At 165 μg, however, a suppression of hemorrhage by the sulfanilamide was observable; became more pronounced at 100 μg; and was obvious at 66 and 33 μg. Thus it would appear that sulfanilamide can substantially abolish the hemorrhage effect of no more than 16 minimum hemorrhage doses (selecting the hemorrhage dose represented by 100 μg).

It is of interest to note that in our previous study on the lethality of the endotoxin[1] it was found that sulfanilamide protected

* During the course of this study a highly purified hemorrhage-producing material was extracted from *Salmonella typhimurium* by the use of anhydrous phenol followed by fractionation of the complex toxic antigen by the acid-formamide procedure of Morgan and Partridge. The minimum hemorrhage dose of this purified material is between .05 and .10 μg. Experiments indicate that sulfanilamide exerts the same anti-hemorrhagic effect on this highly purified toxin at low doses as on the toxin preparation used in the present study.

against from 2 to 10 minimum lethal doses, a sign that the protection afforded by sulfanilamide against the hemorrhage factor parallels the protection afforded against the lethal factor. In protecting against lethality, sulfanilamide nullified the effect of 2.6 mg (2 M.L.D.) to 13 mg (10 M.L.D.).

However, in protecting against hemorrhage, sulfanilamide suppressed the effect of no more than 0.006 mg (about 1 M.H.D.) to 0.1 mg (about 16 M.H.D.). The ratio of M.L.D. to M.H.D. is about 1 : 200, obtained by dividing 1.3 mg (1 M.L.D.) by 0.006 mg (1 M.H.D.). Considering that only one two-hundredth of a lethal dose of the relatively unpurified toxin is required for tumor hemorrhage, an inhibition of, say, 90% of one M.L.D. of the toxin should leave a residual toxicity equivalent to 20 minimum hemorrhage doses. As seen in the table, animals may understandably be protected against the lethal effect of the toxin and yet undergo tumor hemorrhage. This observation constitutes further evidence that the vascular bed of the tumor, whose breakdown is responsible for the hemorrhage, is many times more susceptible to the toxin than is the organism as a whole to the lethal action of the toxin. Shear,[3] using a highly purified bacterial toxin, has reported that the hemorrhage-lethality ratio may be as high as 1 : 5000 if lethality is measured with non-tumor-bearing mice, and 1 : 500 if lethality is measured with tumor-bearing mice.

The mode of action of sulfanilamide in respect to protection against lethality and tumor-hemorrhage is as yet unknown. It is interesting that Andervont and Shimkin[4] found that ascorbic acid affords protection against toxin-induced tumor hemorrhage. It appears difficult to decide, however, whether this effect is mediated by raising the threshold of capillary resistance, or whether ascorbic acid (or sulfanilamide) may participate in a specific detoxication directed against the bacterial endotoxin.

In view of the previous conclusion[2] that the hemorrhage factor is a component of the O antigens of most gram negative bacteria, and in line with the evidence that sulfanilamide inhibits both the lethal effect and the tumor hemorrhage effect of these antigens, it appears reasonable from this parallelism in action that both tumor-hemorrhage and lethality are expressions of a common chemical factor characteristic of the O antigens.

[3] Shear, H. J., *Cancer Research*, 1941, **1**, 731.

[4] Andervont, H. B., and Shimkin, M. B., *Am. J. Cancer*, 1939, **36**, 451.

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Effect of Electrolytes upon Kahn Precipitates from Human and Animal Sera.

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The problems created by the existence of falsely positive serologic reactions have been a source of difficulty since the advent of the Wassermann test.[1] Kahn[2] reported the first satisfactory differential test; this was based upon specific temperature differences.

We became interested in determining whether or not reactions in human and animal sera could be distinguished by the effect of electrolytes. Animal sera were used in these studies because they give general biologic reactions which are apparently of the same type as those found in human falsely positive serologic reactions.[2,3]

[1] Wassermann, A., Neisser, A., and Bruck, C., *Deutsch med. Wchnschr.*, 1906, **32**, 745.

[2] Kahn, R. L., *Arch. Derm. and Syph.*, 1940, **41**, 817.

[3] Eagle, H., *Laboratory Diagnosis of Syphilis*, Chap. XVIII, C. V. Mosby Co., 1937.