known, because it has been obtained from extracts of disrupted, washed organisms.[2, 3] The toxic substance in washings from *H. pertussis* seems to be analogous to that obtained by Zinsser and by Parker[4] from a variety of other organisms and called by them the "X-substance."

In *H. pertussis* washings other components, such as a heat-stable factor, an agglutinogen, and derivatives from the culture medium, are no doubt present in varying amounts. That a specific soluble substance could be removed by washing the organisms with saline or water has been demonstrated by Miller.[5]

The chemical composition, the antigenic properties and the effect of the toxic washings upon blood leucocytes are interesting points now being studied.

Summary. A heat-labile substance, toxic for young mice has been obtained by washing suspensions of freshly isolated strains of *Hemophilus pertussis* with 0.85% NaCl solution. Both the lethal and the skin-reacting factors of the toxic substance can be neutralized by antitoxin but not by antibacterial rabbit sera.

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Non-Specificity of Sulfonamides.

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The notion that individual members of the sulfonamide series are specific for certain bacterial species is widespread, although contrary to the most widely accepted hypothesis of the mode of sulfonamide action. The following experiments serve to clarify this point:

Staphylococci were grown on Knight's medium modified by Fildes, *et al.*,[1] and *Escherichia coli* on the asparagine-glucose-mineral

[2] Evans, E. G., and Maitland, H. B., *J. Path. and Bact.*, 1937, **45**, 715.

[3] Flosdorf, E. W., and Kimble, A. C., *J. Immunol.*, 1940, **39**, 475.

[4] Zinsser, H., Enders, J., and Fothergill, L., *Immunity Principles and Application in Medicine and Public Health*, The Macmillan Co., New York, 1939, 78-101.

[5] Miller, J. J., PROC. SOC. EXP. BIOL. AND MED., 1937-38, **37**, 45.

[1] Fildes, P., Richardson, G. M., Knight, B. C. J. G., and Gladstone, G. P., *Brit. J. Exp. Path.*, 1936, **17**, 481.

TABLE I.
Concentrations of Sulfonamides Permitting ½ Maximum Growth.

	Knight's medium		MacLeod's medium
	Staph. aureus mg%	*E. coli* mg%	*E. coli* mg%
Sulfanilamide	14.0	15.0	5.8
Sulfaguanidine	12.0	16.2	5.8
Sulfapyridine	3.0	2.9	.61
Sulfacetimide	2.0	2.8	.57
Sulfadiazine	.6	.65	.061
Sulfathiazole	.3	.65	.062

salts medium suggested by MacLeod.[2] From a 24-hour culture one million washed cells per ml were inoculated into a flask. The flask was incubated at 37°C for one-half hour to permit adjustment to the conditions of culture before the inoculated medium was dispensed into pyrex tubes containing the drugs. Thus all variations as to strain resistance, size of inoculum, and condition of the medium were eliminated. The tubes were incubated and growth was followed by electrophotometric turbidity measurements.

Table I gives the results of experiments carried out with *Staphylococcus aureus* and *E. coli*. We used as a convenient end-point that concentration of sulfonamide which permitted one-half maximum growth after 16 hours. In this manner the six sulfonamides are divided into pairs according to their effectiveness. This order of potency is not affected by the species of organism or by the medium.

The molecular ratio of the antagonistic pair, *p*-aminobenzoic acid and sulfanilamide, has been the subject of several investigations.[3] It has been pointed out that this ratio is merely a measure of their respective affinity for the unknown receptor site in the organism. If this view is acceptable, then a detailed investigation of these ratios should yield useful information on the general mechanism of sulfonamide action.

Our experiments indicate that these ratios can be used as sensitive and reproducible criteria for the effectiveness of sulfonamides. In such studies one sulfonamide concentration was employed and a range of p-amino benzoic acid concentrations were added to permit estimation of the level restoring growth to the half maximum. Data in support of this procedure are presented in Table II, using one million *E. coli* per ml.

[2] MacLeod, C. M., *J. Exp. Med.*, 1940, **72**, 217.

[3] Woods, D. D., *Brit. J. Exp. Path.*, 1940, **21**, 74; Lockwood, J. S., *Surg. Gynecol. Obstet.*, 1941, **72**, 307; Wyss, O., PROC. SOC. EXP. BIOL. AND MED., 1941, **48**, 122; Strauss, E., Lowell, F. C., and Finland, M., *J. Clin. Invest.*, 1941, **20**, 189; Spink, W. W., and Jermsta, J., PROC. SOC. EXP. BIOL. AND MED., 1941, **47**, 395.

TABLE II.
Effect of Concentration of Sulfonamide on Sulfonamide-*p*-Aminobenzoic Acid Ratio.

Sulfonamide	mg%	P.A.B. mg%*	Molar ratio
Sulfanilamide	200	.08	2000
"	100	.05	1600
"	50	.02	2000
"	25	.010	2000
Sulfathiazole	10	.2	27
"	5	.1	27
"	1	.02	27
"	.3	.007	23

*Concentration required to restore growth to ½ maximum.

The molar ratios of the antagonists are constant for a wide range of concentrations provided that sufficient excess is employed in order that the native sulfonamide inhibitor present in the inoculum does not introduce an appreciable error and provided that the concentration is not so excessive as to affect the physiologic properties of the medium significantly.

The effectiveness of these sulfonamides, determined in terms of their efficiency in overcoming *p*-aminobenzoic acid, is shown in Table III.

From the range of *p*-aminobenzoic acid concentrations employed the level required to restore growth to the half maximum was estimated and the molar ratios of sulfonamide to inhibitor were computed. The similarity of the absolute values (columns 3 and 6) may be fortuitous, but the close agreement of the comparative efficiencies (columns 4 and 7) is highly significant.

A study of this ratio, using two widely differing sulfonamides, was extended to other organisms. *Staphylococcus aureus* and *Proteus vulgaris* were grown on Knight's medium, and *Lactobacillus acidophilus* on a modification of the medium of Snell and Mitchell[4]

TABLE III.
Sulfonamide Efficiency in Knight's Medium.

		Staph. aureus			*E. coli*		
Drug	mg%	PAB mg%*	Molar ratio Drug/PAB	Efficiency	PAB mg%*	Molar ratio Drug/PAB	Efficiency
Sulfanilamide	50	.0086	4660	1	.012	3330	1
Sulfaguanidine	50	.0070	4570	1	.0081	3960	.8
Sulfapyridine	5	.0066	416	11	.0061	450	7
Sulfacetimide	5	.0060	534	9	.0060	534	6
Sulfadiazine	5	.030	92	51	.064	43	78
Sulfathiazole	5	.050	53	88	.065	41	81

*Concentration restoring growth to ½ maximum.

[4] Snell, E. E., and Mitchell, H. K., *Proc. Nat. Acad. Sci. U.S.*, 1941, **27**, 1.

TABLE IV.
Neutralization of *p*-Aminobenzoic Acid by Sulfanilamide and Sulfathiazole.

Test organism	Mol. SA/PAB	Mol. ST/PAB	Efficiency ST/SA
E. coli	2,000	27	74
A. aerogenes	3,220	45	72
Staph. aureus	4,660	53	88
Ps. aeruginosa	13,330	184	73
Sal. typhimurium	6,650	92	72
L. acidophilus	8,000	133	60
Proteus vulgaris	4,000	55	73

to which we added a small amount of ether-extracted yeast-extract in place of the purines. The other organisms were grown on MacLeod's medium. The results in Table IV indicate that the efficiency ratio of the two drugs in counteracting the effect of *p*-aminobenzoic acid is strikingly constant.

These data demonstrate the absence of specificity with respect to any of the 7 organisms. A modification of the experiment outlined in Table IV using *E. coli* and sulfanilamide and sulfathiazole, showed that this relation also obtained when the comparative efficiency was determined by plate counts after 2, 4, and 8 hours' growth. Similarly, if peptone was used as inhibitor, the same efficiency ratio held.

Specificity, of course, can be demonstrated under special conditions. For example, as pointed out by Dorfman, *et al.*,[5] an additional inhibition by sulfapyridine is observed when organisms sensitive to nicotinamide deficiency are studied in a medium low in that growth factor. Differences in solubility characteristics, absorption, penetration, maintenance and excretion are also valid practical characteristics of these derivatives which have an important bearing on the clinical aspects of this problem. Our data, however, refer to the fundamental aspects of the action of sulfonamides upon growing bacteria.

This view is a generalization of one aspect of this problem presented by one of us[3] previously. In a wide variety of organisms, the receptor site in the bacterial cell is the same and the difference in effectiveness between the members of this series is a characteristic of the individual sulfonamide and not the species of organism involved.

Summary. The molar ratio of *p*-aminobenzoic acid to sulfonamide required to restore a sulfonamide-inhibited inoculum to half maximum growth was determined. Using this ratio as a criterion for the effectiveness of the various sulfonamides, no evidence of specificity was found.

[5] Dorfman, A., Rice, L., Koser, S. A., and Saunders, F., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 750.