Summary. Under the conditions of our experiments the protective value of highly concentrated type specific serum in optimal doses and that of sulfapyridine in optimal doses are approximately equal when the infecting dose of Type III pneumococci is relatively small, resulting in a mortality of 63% in untreated animals. When the infecting dose is sufficiently large to produce an initial mortality of 100%, the mortality after sulfapyridine therapy is significantly less than after serum therapy. Combining serum and sulfapyridine, each in optimal dose, does not reduce mortality below that of sulfapyridine therapy alone in Type III pneumococcic pneumonia, differing in this respect from results previously obtained from similar experiments with Type I pneumococcic pneumonia.

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Effect of Sulfanilamide, Sulfapyridine, and Sulfathiazole on Staphylococcus Toxins.*

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Conflicting reports have appeared in the literature on the neutralization of staphylococcus toxins by sulfanilamide and allied compounds. Levaditi and Vaisman[1] were unable to demonstrate any effect of prontosil, neoprontosil, and other azo-sulfonamide derivatives against staphylococcal hemolysin, although they claimed these compounds neutralized the effect of streptococcal leucocidin and hemolysin. Later Levaditi, Vaisman, and Reinie[2] reported that none of the compounds tested was effective against staphylococcus lethal toxin. Osgood and Powell[3] found that sulfanilamide in concentrations of 1:1000 or less did not inactivate significant amounts of staphylococcal hemolysin. Recently Carpenter and his co-

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1 Levaditi, C., and Vaisman, A., *Compt. Rend. Soc. de Biol.*, 1935, **120**, 1077.

2 Levaditi, C., Vaisman, A., and Reinie, L., *Compt. Rend. Soc. de Biol.*, 1937, **126**, 1937.

3 Osgood, E. E., and Powell, H. M., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 37.

workers[4-7] have reported an antitoxic effect of sulfanilamide and its derivatives on toxins formed by the gonococcus, pneumococcus, staphylococcus, *Streptococcus hemolyticus, Clostridium botulinum, Clostridium tetani, Clostridium septicum* (vibrion septique), and *Clostridium perfringens.* Although in his *in vivo* experiments he reports in one paper[5] neutralization, and in another paper,[7] failure to neutralize staphylococcus lethal toxin, he reports statistically valid *in vitro* experiments demonstrating consistently the neutralization of staphylococcus lethal toxin.

In the present study, toxins from 4 strains of hemolytic staphylococci were used, as well as one batch of toxin labelled "Lot O" prepared by Carpenter. The effect of sulfanilamide (para amino benzene sulfonamide), sulfapyridine (2-sulfanilyl amino pyridine), and sulfathiazol (2-sulfanilyl aminothiazole) on these toxin preparations was investigated. The toxins were prepared according to the method of Dolman and Wilson.[8] A 2-day culture of the organism following growth on semi-solid agar was passed through filter paper, and finally through a Seitz filter to remove the bacteria. The M.L.D. of each lot of lethal toxin was determined for adult mice weighing 22 to 26 g. In this study two strains of albino mice and one strain of black mice were used, but only one strain of mice was used in each experiment. Each group, including the control group, contained the same proportion of males and females with comparable weights. The mice were observed for 7 days, although very few deaths occurred after 48 hours. The sulfanilamide or its derivative (in 0.85% sodium chloride solution) was thoroughly mixed with the toxin and 1 cc of the mixture injected intraperitoneally into each mouse from 5 to 45 minutes following mixing. Control mice received 1 cc of the saline solution containing the same amount of toxin.

During initial experiments in which a dose of toxin was administered sufficient to kill 100% of the control mice, it was always found that 100% of the mice injected with toxin-sulfanilamide mixtures also died. Subsequent experiments were therefore per-

[4] Carpenter, C. M., Barbour, G. M., and Hawley, P. L., *J. Pediatrics,* 1939, **14**, 116.

[5] Carpenter, C. M., and Barbour, G. M., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 354.

[6] Carpenter, C. M., and Barbour, G. M., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 255.

[7] Carpenter, C. M., *Proc. Third International Congress for Microbiol.*, 1939, **7**, 595.

[8] Dolman, C. E., and Wilson, R. J., *J. Immunology,* 1938, **35**, 13.

TABLE I.
Effect of Sulfanilamide and Allied Compounds on Staphylococcus Lethal Toxin.

	Mice injected		
	Total	No. survived	% survival
Control	150	79	56
1:100 Sulfanilamide	100	36	36
1:200 "	100	39	39
1:1000 "	120	55	45
1:1000 Sulfapyridine	120	58	48
1:1000 Sulfathiazole	120	61	51

formed in which approximately half of the control mice survived. Table I summarizes the results of the latter experiments. It is evident that rather than neutralizing the lethal action of the toxin, if anything the drugs slightly enhanced the toxicity to mice. It is difficult to reconcile these results with those of Carpenter. It was therefore thought desirable to determine whether these compounds might influence the other toxic activities of staphylococcus toxin.

The neutralization of dermo-necrotic toxin was tested by injecting 4 albino guinea pigs and 4 albino rabbits with staphylococcus toxin plus various dilutions of sulfanilamide and its derivatives. Each animal was injected intradermally with .2 cc of each mixture. The area of necrosis was measured on the fifth day. The average of these measurements is listed in Table II.

It is obvious that there was no neutralization of the dermo-necrotic factors present in the toxin.

The effect on the hemolysins was studied by several methods. Using 2 hemolytic units of toxin with the addition of various concentrations of the sulfonamides to a 1% rabbit erythrocyte suspension, it was found that high concentrations of the chemicals reduced the hemolysis partially, whereas concentrations normally attained in the blood during treatment of infections had no effect. This

TABLE II.
Effect of Sulfonamides on Dermo-necrotizing Toxin.

	Avg of areas of necrosis, cm^2
Toxin + saline solution (control)	2.2
" + 1:10,000 sulfanilamide	2.0
" + 1: 1,000 "	2.2
" + 1: 100 "	2.3
" + 1:10,000 sulfapyridine	2.2
" + 1: 1,000 "	2.1
" + 1:10,000 sulfathiazole	2.2
" + 1: 1,000 "	2.0

TABLE III.
Effect of Sulfonamides on Alpha-Hemolysin.
Rabbit erythrocytes 1% + 2 hemolytic units staphylococcus hemolysin incubated 1 hour 37°C.

Concentration, %	.1	.05	.025	.0125	.006*	.0002*	Control
Sulfanilamide	2+	3+	3+	4+	4+	4+	4+
Sulfapyridine			2+	3+	4+	4+	4+
Sulfathiazole			2+	3+	4+	4+	4+

*Concentrations of .003, .0015, .0008, and .0004 gave same results as .006 and .0002.

confirms the report of Osgood and Powell,[8] and of Gross, Cooper, and Lewis.[9] Similar experiments, using sheep erythrocytes with incubation at 37°C for one hour followed by 12 hours at 7°C, gave essentially the same result.

Blood agar plates were prepared containing various concentrations of the 3 chemicals. The results in Table IV demonstrate that decreased production of hemolysin by staphylococci is due mainly to a decreased growth rate.

In a series of experiments to determine the effect of these compounds upon staphylococcus enterotoxin, no demonstrable neutralizing activity was found. Kittens injected intraperitoneally with toxin-sulfanilamide mixtures vomited in the same length of time, and with the same degree of severity as kittens injected with toxin alone.

Using dilutions of 24 hour broth cultures of various strains of staphylococci mixed with various concentrations of the 3 compounds, no significant alteration of coagulase other than might be attributed to reduced growth rate of the organism was observed.

Although numerous statements have been made concerning the mechanism by which sulfanilamide acts, there is very little proof in support of any of these, other than those concerning its bacteriostatic action. The experiments described in this report were performed in the hope that the neutralization of the toxic products

TABLE IV.
Effect of Sulfonamides on Hemolysin Production in Blood Agar Plates.

	Control	Sulfanilamide			Sulfapyridine		Sulfathiazole	
Concentration %		.01	0.1	1.0	.01	0.1	.01	0.1
Diameter hemolysis (mm)	4.5	3.3	2.1*	0.3*	2.8*	2.2*	2.8*	1.2*
Diameter colony (mm)	1.6	1.5	1.0	0.2	0.8	0.7	1.0	0.7

*Indicates partial hemolysis.

[9] Gross, P., Cooper, F. B., and Lewis, M., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 275.

might be one of the main activities of the sulfonamide compounds, and that more potent antitoxic compounds might prove even more useful in the treatment of bacterial infections. These experiments, however, lead one to believe that the only manner in which the toxicity of staphylococci is affected is by an inhibition of growth of the organism with a consequent decreased production of toxin.

Summary. Toxic manifestations of staphylococci are not inactivated *in vitro* by sulfanilamide, sulfapyridine, or sulfathiazole. The lethal toxin, dermo-necrotic toxin, coagulase, and enterotoxin are not neutralized by solutions of the sulfonamides tested at 37°C. α- and β-hemolysins are slightly diminished in activity at concentrations approaching the saturation point of the sulfonamides, but are unaffected at concentrations of less than .01%. These compounds appeared to decrease hemolysin production by decreasing the growth rate of the organism.

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Response of Plasma Volume to Diuretics.

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Previous work[1] has led us to conclude that mercurial diuretics act by diminishing tubular reabsorption, while administration of aminophyllin produces an increase in the volume of the glomerular filtrate. Earlier work[2] suggested a high circulating blood volume in congestive heart failure, but there was no complete agreement as to the change following diuresis. Recent determinations[3] by a more satisfactory method[4] demonstrate a marked elevation of blood and plasma volume in patients with failure, and a decrease towards normal with the development of circulatory compensation. A similar decrease has been noted after the use of mercurial diuretics.[5]

We have followed over 12 to 24 hours the changes in plasma volume, determined by the method of Gibson and Evelyn,[4] after the

1 Herrmann, G., and Decherd, G., *J. Lab. and Clin. Med.*, 1937, **22**, 767.

2 Goldhammer, S., Leiner, G., and Scherf, D., *Klin. Woch.*, 1935, **14**, 1109.

3 Gibson, J. G., and Evans, W. A., *J. Clin. Invest.*, 1937, **16**, 851.

4 Gibson, J. G., and Evelyn, K., *J. Clin. Invest.*, 1938, **17**, 153.

5 Harris, Alfred W., personal communication.