in causing this reversibility, when 0.01 or even 0.001 mg of the substance was added to the tubes containing previously swollen capsules. The specificity of this reversibility was indicated by the fact that addition of Type I polysaccharide failed to reverse the reaction.

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Effect of Sulfapyridine, Sulfathiazole and Sulfamethylthiazole upon Severe Staphylococcal Infection in Mice.*

ELEANOR A. BLISS† AND EARL OTT. (Introduced by P. H. Long.)

From the Biological Division of the Department of Medicine, The Johns Hopkins University Medical School and The Johns Hopkins Hospital, Baltimore, Maryland.

In previous reports[1, 2] it was shown that while sulfanilamide therapy had a slight effect upon the course of staphylococcal infections in mice, the use of sulfapyridine markedly prolonged the lives of the infected animals. In a few instances the mice treated with sulfapyridine survived 2 and 3 months.[3] Eventually, however, they succumbed, showing at postmortem, abscess formation in the kidneys and liver.

Preliminary studies[4, 5] in which the *in vitro* effect of sulfathiazole was tested, indicated that this compound inhibited the growth of a number of microörganisms, including staphylococci, to a greater degree than did sulfanilamide and sulfapyridine. It was then considered worthwhile to compare the activity of this compound and of sulfapyridine in the treatment of severe staphylococcal infections in mice. Later the activities of sulfathiazole and sulfamethylthiazole were similarly compared.

Methods. The mice were infected by the intravenous injection of heavy broth suspensions of a strain of *Staphylococcus aureus* which had been isolated a year previously from a patient, ill with a lung abscess. The method is given in detail elsewhere.[1]

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† Fellow in Medicine, The Johns Hopkins University Medical School, on a grant from the Calco Division of the American Cyanamid Company.

1 Feinstone, W. H., Bliss, E. A., Ott, E., and Long, P. H., *Bull. Johns Hopkins Hosp.*, 1938, **62**, 565.

2 Bliss, E. A., and Long, P. H., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 32.

3 Unpublished observations.

4 Lawrence, C. A., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 92.

5 Long, P. H., and Bliss, E. A., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 324.

TABLE I.

The Effect of Diets Containing 1% Sulfapyridine, 1% Sulfathiazole or 1% Sulfanethylthiazole upon the Survival Time of Mice Infected with *Staphylococcus aureus*.

	*Series A			†Series B			Series A		Series B	
	No. of mice			No. of mice			No. of mice			
	30	49	50	19	30	30	20	20	30	30
	Controls	Sulfapyridine	Sulfathiazole	Controls	Sulfathiazole	Sulfamethylthiazole	Sulfapyridine	Sulfathiazole	Sulfathiazole	Sulfamethylthiazole
Day of experiment	No. of mice dying						Avg daily consumption of drug—mg/mouse			
—2	(before infection)						not measured		20.2	18.9
—1							22.5	22.4	26.6	24.7
1	14	11	4	3	0	0	6.5	14.6	14.2	14.3
2	1	3	4	0	2	1	21.7	31.6	23.5	22.0
3	4	1	1	7	1	1	23.2	26.1	29.5	23.9
4	3	1	0	4	0	3	29.9	30.5	28.7	29.3
5	2	3	1	2	1	1	30.1	30.6	31.2	30.5
6	0	1	1	1	0	0	27.6	30.8	28.2	31.2
7	3	4	2	2	1	0	not measured		29.0	27.8
8	2	9	1		1	0			32.5	28.3
9	0	5	10		0	1			29.3	30.6
10	0	3	7		0	2			26.1	29.3
11	0	6	3		1	0			25.2	23.2
12	0	1	1		0	0			26.6	27.7
13	0	1	10		1	2			25.4	24.5
14	1		2		1	1			27.3	26.4
15			1		1	2				
16			0		4	1	Avg per mouse/day			
17			0		1	1	23.0	26.6	26.8	25.6
18			0		1	5				
19			0		1	3				
20			1		6	1				
21			0		3	1				
22			0		1	1				
23			0		0	2				
24 to 30			0		0	0				
Survivors (30 days)	0	0	1	0	3	1				
Mean survival time (days)	2.86	5.86	9.04	3.18	16.03	14.20				
Median survival time (days)	2.0	7.0	9.1	2.9	17.0	16.0				
σ	3.07	3.78	5.19	1.7	7.46	6.90				
Standard error of mean	0.56	0.54	0.74	0.38	1.36	1.26				
Standard error of median	0.70	0.68	0.93	0.49	1.70	1.58				

*Series A is a summary of 3 experiments started on 12/13 and 14/39 and 1/5/40. The mice received 2 billion, 175 million and 2 billion cocci on the respective dates. Therapy maintained 7 days after infection. Drug consumption was determined only in last experiment.

†Series B is a summary of 2 experiments started on 1/25 and 26/40. The mice received 2 billion and 6 billion cocci on the respective dates. Therapy maintained for 2 wks after infection. Drug consumption was determined in both experiments.

Treatment was carried out by administering the drug in the animals' diet. This method was selected because it is the simplest and at the same time, as McKee and her associates pointed out,[6] the surest means of maintaining a fairly even concentration of these drugs in the blood of the mice. The diet consisted of well ground Purina Dog Chow. The drug was added to this in an amount sufficient to give a 1% concentration and the two were mixed carefully and thoroughly. The mice were kept in individual cages. The drug-diet mixture, offered in containers as described by Bieter, *et al.*,[7] was weighed daily in order to ascertain the amount which had been consumed. Treatment was carried out in this way for 2 days prior to infection and, in the first series of experiments, for one week thereafter, in the second for 2 weeks.

Results. The results are shown in Table I. The mice consumed approximately equal quantities of the 3 drug-diets. In the first series of experiments the mean survival times were 2.86 days for the untreated animals, 5.86 days for those treated with sulfapyridine and 9.04 days for those treated with sulfathiazole. These differences are statistically significant. When the median survival times are calculated the difference between the survival times of the sulfathiazole and sulfapyridine-treated animals (9 and 7 days respectively) is less obviously significant. Sulfathiazole and sulfamethylthiazole, as shown in the second series of experiments, were almost equally effective in prolonging the lives of the infected animals. The difference in the results with sulfathiazole in the two experiments is, of course, due to the fact that therapy was maintained twice as long in the second series. It is interesting that in both series the average mouse treated with sulfathiazole survived just 2 days after treatment was discontinued.

Discussion. These results are in accord, as far as the comparison of sulfathiazole and sulfapyridine is concerned, with those of Barlow and Homburger,[8] when allowance is made for the differences in the two technics. They do not agree with these authors' results with sulfathiazole and sulfamethylthiazole since they found sulfamethylthiazole to be the more effective of the two compounds, and we found no therapeutic difference between them. The superiority of sulfamethylthiazole, in the hands of Barlow and Homburger, is probably

[6] McKee, C. M., Rake, Geoffrey, Greep, R. O., and van Dyke, H. B., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 417.

[7] Bieter, R. N., Larson, W. P., Cranston, E. M., and Levine, Milton, *J. Pharm. and Exp. Therap. Proc.*, 1939, **65**, 3.

[8] Barlow, O. W., and Homburger, E., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 792.

attributable to the fact that after the first 32 hours in their experiments the drugs were given in single daily doses and as sulfathiazole is excreted more rapidly than its methyl derivative, the blood levels obtained with it would have a lower daily average.

Summary and Conclusions. Sulfathiazole and sulfamethylthiazole, administered as 1% of the diet showed a distinct and equal therapeutic value in prolonging the lives of mice heavily infected with *Staphylococcus aureus.* Sulfathiazole proved to be somewhat more efficient in this respect than sulfapyridine.

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Effect of Pregneninolone (17-Ethinyl Testosterone) on Genital Tract of Immature Female Rats.

U. J. SALMON AND A. A. SALMON.

From the Laboratories of the Mount Sinai Hospital, New York City.

It has previously been shown that testosterone, when administered to immature female rats, exhibits 3 biological properties: (a) it is gynecogenic, causing premature opening of the vagina[1] and growth of the epithelial and muscular elements in both the vagina and uterus;[2, 3] (b) it is androgenic, causing growth of the clitoris and preputial glands;[2, 3] (c) it is hypophyseotropic, stimulating the hypophysis to secrete gonadotropic hormone, which is manifested by growth of follicles and appearance of corpora lutea in the ovaries.[3-7]

1 Butenandt, A., and Kudzus, H., *Hoppe-Seyler's Z.*, 1935, **75**, 237.

2 Korenchevsky, V., Dennison, M., and Hall, K., *Biochem. J.*, 1937, **31**, 780.

3 Salmon, U. J., *Endocrinology*, 1938, **23**, 779.

4 Salmon, U. J., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 352.

5 Nathanson, I. T., Franseen, C. C., and Sweeney, A. R., Jr., PROC. SOC. EXP BIOL. AND MED., 1938, **39**, 385.

6 Starkey, W. F., and Leathem, J. H., PROC. SOC. EXP. BIOL. AND MED., 1938 **39**, 218.

7 Freed, S. C., Greenhill, J. P., and Soskin, S., PROC. SOC. EXP. BIOL. AND MED. 1938, **39**, 440.