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Protective Effect of Sulfapyridine, Sodium Sulfapyridine, and Sulfanilamide in Pneumococcus Infection Types I, II, and III.

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The successful clinical use of sulfapyridine in the treatment of pneumonia was stimulated by Whitby's experiments on mice. Whitby[1] was able to prolong the life of mice inoculated with various types of pneumococci for 7 days, while with sulfanilamide the average survival was 1.3 to 3.3 days.

Fleming[2] found that sulfapyridine *in vitro* retards the growth in human blood of pneumococci in concentrations which it is reasonably supposed can be obtained therapeutically. MacLean, Rogers, and Fleming[3] found that the sensitivity to sulfapyridine of different strains belonging to the same type of pneumococcus varied considerably. Types I, II, and III were most resistant.

In the experiments of Cooper, Gross and Lewis[4] sulfanilamide-treated mice showed a survival of 25%; with sulfapyridine 45% survived. Our results are more in accord with those of Long, Bliss, and Feinstone,[5] who reported that with a highly virulent strain of pneumococcus type I they obtained 100% mortality with sulfanilamide, and 88% mortality when sulfapyridine was used.

Chemistry. Sulfapyridine is closely related to sulfanilamide, a hydrogen of the amido group being replaced by the pyridyl group. Its chemical properties were described by us in a previous paper.[6] The sodium salt of sulfapyridine was first described by Marshall, Bratton, and Litchfield.[7] This product was prepared independently by us. It is a colorless, crystalline substance, easily soluble in water, insoluble in organic solvents.

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1 Whitby, L. E. H., *Lancet*, 1938, **1**, 1210.

2 Fleming, A., *Lancet*, 1938, **2**, 74.

3 MacLean, I. H., Rogers, K. B., and Fleming, A., *Lancet*, 1939, **1**, 562.

4 Cooper, F. B., Gross, P., and Lewis, M., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 37.

5 Long, P. H., Bliss, E. A., and Feinstone, W. H., *Pa. Med. J.*, 1939, **42**, 483.

6 Raiziss, G. W., Severac, M., Moetsch, J. C., and Clemence, L. W., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 434.

7 Marshall, E. K., Jr., Bratton, A. C., and Litchfield, J. T., Jr., *Science*, 1938, **88**, 597.

TABLE I.
Toxicity of Sulfanilamide, Sulfapyridine, and Their Derivatives in Rabbits *Per Os* and Intravenously.

Drug	Dose per kilo (g)	No. of animals	Survivals		Deaths	
			No.	%	No.	%
Sulfapyridine*	1.5	7	7	100		
	2.0	6	4	66⅔	2	33⅓
Sulfanilamide*	1.5	18	17	94	1	6
	2.0	16	8	50	8	50
Sodium sulfapyridine†	0.5	3	3	100		
	0.75	3	1	33⅓	2	66⅔
	1.0	2			2	100
Sodium sulfanilamide†	0.2	1	1	100		
	0.75	1	1	100		
	1.0	2	2	100		
	1.25	2			2	100
	1.5	1			1	100

Per os. †Intravenously.

Toxicity. 100% of rabbits tolerated 1.5 g of sulfapyridine per kilo given orally, while 66% survived a dose of 2 g per kilo; 94% tolerated 1.5 g and 50% tolerated 2 g of sulfanilamide per kilo. Sulfapyridine is, therefore, slightly less toxic than sulfanilamide. Intravenously, the sodium salt of sulfapyridine was tolerated in a dose of 0.5 g per kilo, compared to 1 g per kilo for sodium sulfanilamide. Sulfanilamide, therefore, is the less toxic of the two drugs intravenously. (Table I.)

Therapeutic effectiveness in pneumococcus types I, II, and III. Two strains of each of the 3 types of pneumococcus were used. Strain 37 of type I pneumococcus, 1599, type II, and 1460, type III, were obtained from Sharp and Dohme.* Strain M30, type I, M48, type II, and M50, type III pneumococcus were supplied through the courtesy of Dr. Perrin Long, of Johns Hopkins Hospital, who considered them very virulent. We found the Sharp and Dohme strains equally as virulent. These strains usually killed mice in a dilution of 10^{-7} (1:10,000,000) to 10^{-8} (1:100,000,000). Mice were infected intraperitoneally with 200 minimum lethal doses of the culture. Many individual experiments were made, each compound being tested on 5 or 10 mice. In Table II are presented summaries of the results obtained in these individual tests. Pneumococci types I, II, or III were subjected to 3 different systems of testing. In the first system only 5 treatments of the drug were given, in the second 10, and in the last a maximum of 18 (Long's method). Further modifi-

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TABLE II.

Therapeutic Effect in Types I, II, and III Pneumococcic Infection in Mice.
Each treatment consisted of 0.010 g of the drug by mouth.

Type	Strain	Drug	No. of mice	Percentage of survivals in days									
				1	2	3	4	5	6	7	14	21	28
I	37†	Sulfapyridine	20	100	90	45	30	15	5	5	5	5	5
		Sulfanilamide	10	100	70	20	0						
		Sodium sulfapyridine	5	100	100	100	60	0					
	M36†	Sulfapyridine	25	96	80	12	4	4	0				
		Sulfanilamide	15	100	47	0							
		Sodium sulfapyridine	15	100	93	47	33	27	27	27	20	20	13
	M36‡	Sulfapyridine	10	100	100	50	30	30	20	20	10	10	10
		Sulfanilamide	10	30	0								
		Sodium sulfapyridine	10	100	90	80	80	80	60	60	40	30	30
II	1599*	Sulfapyridine	30	100	70	30	23	13	3	0			
		Sulfanilamide	15	80	0								
	1599†	Sulfapyridine	70	100	100	61	21	14	8	7	7	7	7
		Sulfanilamide	45	89	25	0							
		Sodium sulfapyridine	10	100	90	80	20	10	10	0			
	M48‡	Sulfapyridine	20	100	95	75	30	22	17	8	2.5	0	
		Sulfanalimide	20	90	68	13	5	0					
		Sodium sulfapyridine	15	100	100	85	60	53	47	3	0		
III	1460†	Sulfapyridine	30	84	70	37	23	23	20	20	20	20	20
		Sulfanilamide	20	70	30	10	5	5	5	5	5	5	5
		Sodium sulfapyridine	15	100	93	47	33	20	20	20	20	20	20
	M50†	Sulfapyridine	5	100	80	20	0						
		Sulfanilamide	5	80	0								
		Sodium sulfapyridine	5	100	80	40	0						
	M50‡	Sulfapyridine	10	100	100	80	60	40	40	40	10	0	
		Sulfanilamide	10	90	30	10	0						
		Sodium sulfapyridine	10	100	100	100	60	40	40	30	20	20	20

*Treated 1½ hr after infection, and once daily until total of 5 treatments. †Treated 1½ and 6 hr after infection first day, twice on second day, and once daily until treatments total 10. ‡Treated immediately, 6 and 12 hr after infection, three times daily for 4 days, twice daily for one day, and once on the last day.

cations concerned the time of treatment, that is, immediately following infection or 1½ hours after, also the number of daily treatments, namely, once, twice or 3 times a day. The dose in every treatment has been invariably 10 mg.

Pneumococcus type I. When strain 37 was used sulfapyridine in the first 5 days was effective in prolonging life, but on the sixth day only 5% of mice survived. This shows then that sulfapyridine prolongs the life of mice, but cannot prevent death. Sulfanilamide kept 20% of infected mice alive for 3 days; on the 4th day all were dead. Sodium sulfapyridine showed a good therapeutic effect until the 4th day, 60% of mice surviving, but all died on the fifth day.

All mice treated with sulfanilamide were dead on the third day when strain M36 was used. Of the sulfapyridine-treated mice, 80% on the second day, but only 12% on the third, survived; by the sixth day all animals were dead. With sodium sulfapyridine, 93% of the mice lived for 2 days, 47% for 3 days, and 13% for 28 days.

Substantially better results were obtained for sulfapyridine when the same strain, M36, was used with more intensive treatment, starting immediately after infection; 10% of mice remained alive at the end of the period of observation. Sulfanilamide-treated mice were dead on the second day. Comparatively good results were obtained with sodium sulfapyridine, 60% of mice surviving 7 days, and 30% living at the end of 28 days.

Pneumococcus type II. Of the 30 mice infected with pneumococcus type II, strain 1599, and treated 1½ hours after infection with sulfapyridine, only 23% survived after the fourth day. After 6 days only 3% were alive. No animals treated with sulfanilamide were living at the end of 2 days.

In mice infected with the same strain (1599), but given 10 treatments instead of 5, the effect of sulfapyridine was a little better after 6 days, 8% of animals surviving instead of 3%; 7% survived 28 days. While there is a slight improvement due to the longer treatment, the difference in the therapeutic result is comparatively small. With sulfanilamide, all animals were dead on the third day. Sodium sulfapyridine had about the same effect as sulfapyridine, except that after 6 days of observation all animals were dead.

When mice were infected with strain M48, and treated more intensively than in the previous series of experiments, starting immediately after infection, sulfapyridine showed 17% survival after 6 days. However, after the 14th day, most of the animals began to die, so that on the 21st day none were alive. The intensive treatment did not materially change the therapeutic effect. Sulfanilamide

caused a prolongation of life, although it was small; all animals died by the 5th day. Sodium sulfapyridine kept more animals alive for six days, but on the 7th day only 3% survived.

Pneumococcus type III. Of mice infected with strain 1460 and treated 1½ hours after infection with sulfapyridine, 20% survived on the sixth day; the same number survived on the 28th day. Sulfanilamide also showed better results, 5% of mice surviving 28 days. Sodium sulfapyridine showed the same survival rate as sulfapyridine.

When strain M50 was used, results were inferior to those obtained with strain 1460. All mice were dead on the 4th day with sulfapyridine, on the 2nd day with sulfanilamide, and on the 4th day with sodium sulfapyridine.

In another series of experiments animals infected with strain M50 were treated intensively by Long's method. Treatment was given immediately after infection, 6 and 12 hours later the 1st day, 3 times daily for the next 4 days, twice for one day, and once on the last day. Of mice treated with sulfapyridine, 40% were living on the sixth day, but after 14 days almost all were dead. All sulfanilamide-treated mice were dead on the 4th day. Sodium sulfapyridine showed better results than sulfapyridine, 20% of mice surviving on the 28th day.

Blood cultures. Blood was taken from the tail of mice infected with pneumococcus type I and subcultured on blood agar plates. In

TABLE III.
Presence of Pneumococcus Type I in Blood Cultures* of Mice Treated with Sulfapyridine and Allied Products.

Drug	Mouse Number	Hours after treatment 24	48	72	After 5 days	Heart culture
Sulfanilamide	4	pos.	died			positive
	5	"	"			"
Sulfapyridine	1	"	pos.	died		"
	2	"	"	"		
	3	"	"	pos.	pos.	
	4	"	"	died		"
	5	"	"	pos.	"	
Sodium sulfapyridine	1	neg.	neg.	died		
	2	"	"	pos.	neg.	
	3	pos.	"	neg.	"	
	4	"	pos.	"	"	
	5	neg.	neg.	"	"	
Controls	1	pos.	pos.	died		
	2	"	"	"		

*Routinely the blood for cultures was taken from the tail of the animal; in case of death the blood from the heart was cultured.

case of death, the blood from the heart was cultured. In mice treated with sulfapyridine, the blood was positive throughout 5 days of examination. Pneumococci were present in the blood of mice after 24 hours' treatment with sulfanilamide; after 48 hours mice were dead, and the heart culture proved positive for pneumococci. Mice receiving sodium sulfapyridine, however, showed a number of negative results for pneumococci during 5 days of observation. (Table III.)

Conclusions. 1. Sulfapyridine administered orally to rabbits is slightly less toxic than sulfanilamide. 2. Sodium sulfapyridine given intravenously is considerably more toxic than sodium sulfanilamide. 3. While the protective effect of sulfapyridine in pneumococcal bacteremia types I, II, and III is small, 20 to 30% of mice surviving on the 4th day and less than 10% on the 7th day, it is somewhat higher than that afforded by sulfanilamide, which results in an average survival of 2 to 3 days. Sulfapyridine prolongs life of infected mice only for an additional period of 2 to 4 days. The protective effect of sodium sulfapyridine is of the same order as that of sulfapyridine.

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Monophasic Action Currents from the Uninjured Turtle Ventricle.

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Monophasic action currents obtained by derivation from 2 uninjured points on heart muscle have often been mentioned in the literature. Samojloff,[1] for example, produced a block by transverse cutting or compression of the frog heart and recorded monophasic action currents by derivation from uninjured base and apex. In this experiment, however, the apical muscle constitutes an extension of the lead to the injured surface. DeBoer[2] has recently stressed his observation that, employing base-apex leads in the frog, the weak beat in alternation may be associated with monophasic action currents. Although no injury was produced in these experiments by mechanical means, the blocking of the impulse must have been due

1 Samojloff, A., *Arch. f. d. exp. Physiol.*, 1914, **155**, 471.

2 De Boer, S., *Cardiologia*, 1938, **2**, 292.