11174

Response of Different Strains of Type I Pneumococcus to Sulfapyridine.

L. H. SCHMIDT AND CAROLYN HILLES. (Introduced by S. Tashiro.)

From the Christ Hospital Research Institute and the Department of Biochemistry, College of Medicine, University of Cincinnati, Cincinnati, Ohio.

Studies on the effectiveness of sulfapyridine therapy in experimental infections with type I pneumococcus have given conflicting results. Some investigators[1-5] have found that sulfapyridine has a marked curative action in such infections, while others[6-9] have reported that the drug has little or no curative action and at best merely prolongs life. It has been suggested[3, 7] that these conflicting observations may have been due to the use of different strains of pneumococcus. Some support for this suggestion has been offered by the *in vitro* experiments of MacLean, Rogers and Fleming,[10] which showed that strains of pneumococcus differed in their sensitivity to sulfapyridine; however, the strength of this support has been weakened by the recent observation of Long and Bliss,[11] that 2 strains of type I pneumococcus which reacted quite differently to sulfapyridine *in vitro,* responded similarly to the drug *in vivo.* Since there has been no extensive study of the variations in sensitivity of different strains *in vivo,* it seemed desirable to determine whether such variations occur, and if so, whether certain strains are completely resistant to sulfapyridine.

The present report concerns a study of the effectiveness of sulfapyridine therapy in experimental infections with 12 strains* of

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

2 Schmidt, L. H., and Hilles, C., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 611.

3 Greey, P. H., MacLaren, D. B., and Lucas, C. C., *Canad. Med. J.*, 1939, **40**, 319.

4 MacLeod, C. M., and Daddi, G., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 69.

5 Powell, H. M., and Chen, K. K., *J. Pharm. and Exp. Therap.*, 1939, **67**, 79.

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

7 Bliss, E. A., Feinstone, W. H., Garrett, A. W., and Long, P. H., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 619.

8 Marshall, E. K., personal communication.

9 Raiziss, G. W., Severac, M., Moetsch, J. C., and Clemence, L. W., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 12.

10 MacLean, I. H., Rogers, K. B., and Fleming, A., *Lancet,* 1939, **2**, 563.

11 Long, P. H., and Bliss, E., *Ann. Int. Med.*, 1939, **13**, 232.

* Cultures of pneumococci were obtained through the kindness of the following persons: strains Neufeld, NYS 55, NYS 23 and NYS 63 from Dr. Julia Coffey, New York State Department of Health, Albany; strain Felton from Dr. E. K.

type I pneumococcus—identical experiments being performed with each of the strains except strain Neufeld. Three groups of white mice were infected, intraperitoneally, with approximately 100 lethal doses of a given strain. The mice in one of these groups served as untreated controls. Those in the other groups (A and B) were treated with sulfapyridine according to the schedules† shown in Table I, the drug being administered, orally, as a 10% suspension in 10% acacia. On the tenth day, the surviving mice were divided into 2 groups, one group being kept under observation for an additional 20 days,‡ the other being used in the reinfection experiments described below.

The results of these therapeutic experiments (Table I) show that there were marked quantitative variations in the response of different strains of type I pneumococcus to sulfapyridine. Although these variations were evident in both the Group A and Group B experiments, they were particularly marked in the Group A experiments—those in which the mice received intensive treatment only during the first day and moderate therapy thereafter.§ This treatment was most effective against strain Neufeld, 98% of the mice surviving, and was least effective against strain McGovern, only 23% of the mice surviving. Two of the other strains (M 36 and NYS 55) responded to the drug nearly as well as did strain Neufeld. The remaining 8 strains responded more like strain McGovern, 24 to 43% of the mice surviving infection.

Although moderate sulfapyridine treatment (Group A experiments) was highly effective against infections with strains Neufeld, NYS 55 and M 36, only when intensive treatment was maintained throughout the 6-day period was the drug highly effective against

Marshall, Jr., School of Medicine, Johns Hopkins University, Baltimore, Md.; strain M 36 from Dr. M. Severac, Dermatological Research Laboratories, Philadelphia, Pa.; and strains Lederle 6, Bonner, McGovern, Lockhart, Chauncey and Clement from Dr. Raymond Libby, Lederle Laboratories, Inc., Pearl River, N. Y. The technique used in preparing these pneumococci for therapeutic experiments has been described elsewhere.[12]

† These schedules were based on our earlier work[2] which showed that the treatment given to Group A sufficed to cure a majority of mice infected with highly susceptible types of pneumococci; that given to Group B was effective against infections with moderately susceptible types, but only prolonged life in infections with such resistant types as II, III and VIII.

‡ In this group no deaths occurred later than 10 days after infection.

§ Using a slight modification of Marshall's method[13], it was found that the treatment given to Group A mice maintained a minimum level of 16 mg % free sulfapyridine during the first 24 hours of the experiment and a minimum level of 2 mg % thereafter. The treatment given to Group B mice maintained a minimum level of 16 mg % throughout the entire experiment.

TABLE I.
Summary of Therapeutic and Reinfection Experiments.

	Therapeutic experiments												Reinfection experiments				
	Infecting dose				No. of deaths Days after infection							10-day survivors	Infecting dose			Survivors 20 days after reinfection	
Strain of Pneumococcus	cc 12-hr cult.	No. of colonies	Group	No. of mice in group	1	2	3	4	5	6	7 to 10	No.	%	cc 12-hr cult.	No. of colonies	No. of mice reinfected	No.	%
Neufeld	10^{-6}	160	Control	20	10	10	0	0	0	0	0	0	0	10^{-6}	130	10*	0	0
			A	50	0	0	0	0	1	0	0	49	98			25	21	84
Felton	10^{-6}	130	Control	40	30	10	0	0	0	0	0	0	0	10^{-7}	60	10*	0	0
			A	70	1	1	1	13	29	7	1	17	24			10	2	20
			B	30	0	0	0	1	0	0	2	27	90			15	3	20
NYS 55	10^{-6}	150	Control	20	5	7	8	0	0	0	0	0	0	10^{-6}	150	10*	0	0
			A	30	0	1	0	0	0	0	11	18	60			10	7	70
			B	30	0	0	0	0	0	0	0	30	100			14	13	93
NYS 63	10^{-6}	1940	Control	20	3	7	10	0	0	0	0	0	0	10^{-6}	1210	10*	0	0
			A	30	0	0	0	0	0	0	20	10	33			5	3	60
			B	30	0	0	0	0	0	3	1	26	87			13	13	100
NYS 23	10^{-6}	110	Control	20	4	8	8	0	0	0	0	0	0	10^{-6}	140	10*	0	0
			A	30	0	0	0	2	0	1	17	10	33			5	3	60
			B	30	0	0	0	6	1	0	2	21	70			10	10	100
M 36	10^{-6}	120	Control	30	25	5	0	0	0	0	0	0	0	10^{-6}	120	10*	0	0
			A	30	0	1	5	2	0	0	0	22	73			10	0	0
			B	50	0	0	0	1	1	0	3	45	90			23	3	13
Chauncey	10^{-5}	890	Control	20	0	13	7	0	0	0	0	0	0	10^{-5}	960	10*	0	0
			A	30	0	0	0	0	0	1	16	13	43			7	3	43
			B	30	0	0	0	1	0	0	4	25	83			13	11	85

Lederle 6	10^{-7}	60	Control	20	9	9	2	0	0	0	0	0	0	10^{-7}	60	10*	0	0
			A	30	0	0	0	1	8	1	11	9	30			5	2	40
			B	30	0	0	0	0	0	0	1	29	97			14	2	14
McGovern	10^{-7}	70	Control	20	15	4	1	0	0	0	0	0	0	10^{-7}	40	10*	0	0
			A	30	0	0	0	11	2	3	7	7	23			3	1	33
			B	30	0	0	0	1	1	2	3	23	77			12	10	83
Bonner	10^{-6}	90	Control	20	5	12	3	0	0	0	0	0	0	10^{-6}	160	10*	0	0
			A	30	0	0	0	0	0	0	18	12	40			6	1	17
			B	30	0	0	0	0	0	0	4	26	87			13	8	62
Lockhart	10^{-6}	300	Control	20	0	16	4	0	0	0	0	0	0	10^{-6}	240	10*	0	0
			A	30	0	0	0	0	1	1	19	9	30			5	3	60
			B	30	0	0	0	1	2	2	1	24	80			10	9	90
Clement	10^{-5}	2300	Control	20	2	14	4	0	0	0	0	0	0					
			A	30	0	0	0	0	2	4	16	8	27					
			B	30	0	0	0	1	0	0	12	17	57					

* New controls—not infected previously.
Group A mice received 20 mg of sulfapyridine 2, 8, 14 and 22 hours after infection and every 24 hours thereafter for 5 days.
Group B mice received 20 mg of sulfapyridine 2, 8, 14 and 22 hours after infection and every 8 hours thereafter for 5 days.

the other 9 strains. Under such intensive treatment§ (Group B experiments) 57 to 100% of the mice survived infection, and in only one experiment—that with strain Clement—was the recovery less than 70%. This observation, that the majority of strains of type I pneumococcus responded well only to prolonged intensive treatment, probably explains some of the earlier conflicting reports on the effectiveness of the drug. The superior results of the Group B experiments, as compared with those of Group A, reëmphasize the importance of administering sulfapyridine at frequent and regularly spaced intervals so as constantly to maintain effective levels of the drug in the blood.[8, 12]

It is evident from the data in Table I that the variations in the response of different strains to sulfapyridine were not related to the size of the infecting dose nor to the virulence of the organisms. It is also apparent that the variations were not related to the invasiveness of the strains, as had been suggested previously,[7] for the drug was equally effective against strains of high and low invasiveness. It occurred to us that the effectiveness of sulfapyridine might be related to the capacities of the strains to stimulate production of antibodies, since the antigenicity of strains of pneumococcus varies considerably and recovery from pneumococcal infections generally depends on antibody production. Such a possibility seemed to be supported by MacLeod's observation[14] that the effectiveness of sulfapyridine against Types I and III pneumococci was proportional to the antigenicity of these types.

In order to test this idea we have determined whether the mice that survived the therapeutic experiments were equally immune to reinfection. Ten days after the initial infection (4 days after termination of treatment) half of the surviving mice from Groups A and B were reinfected with approximately the same number of pneumococci (homologous strain) as in the original experiment—suitable controls being reinfected simultaneously.

The results of these experiments (Table I) show that the immunity of sulfapyridine-treated mice to reinfection depends to a great extent on the strain of pneumococcus used as the infecting agent.‖ Mice infected with 8 of the strains (Neufeld, NYS 55, NYS 63, NYS 23, Chauncey, McGovern, Bonner and Lockhart) were highly

12 Schmidt, L. H., and Hilles, C., *J. Infect. Dis.*, 1939, **65**, 273.

13 Marshall, E. K., *J. Biol. Chem.*, 1937, **122**, 263.

14 MacLeod, C. M., *J. A. M. A.*, 1939, **113**, 1405.

‖ This observation provides an explanation for conflicting reports[1, 15, 16] on the immunity of sulfapyridine-treated mice to reinfection.

immune to reinfection, 62 to 100% of the Group B mice surviving. Mice infected with the 3 remaining strains (Felton, M 36 and Lederle 6) had little immunity to reinfection. Only 5 of 25 mice (Groups A and B) reinfected with strain Felton, 3 of 33 reinfected with M 36, and 4 of 19 reinfected with Lederle 6 survived.

A comparison of these results with those of the therapeutic experiments shows that there is little correlation between the effectiveness of sulfapyridine and immunity to reinfection. Thus the drug was equally effective against strains Felton, Lederle 6, McGovern, Bonner, and Lockhart, but mice that survived infection with the first 2 strains had little immunity to reinfection, whereas those surviving infection with strains McGovern, Bonner, and Lockhart were highly immune.

It is evident, therefore, that differences in the antigenicity of the strains were not primarily responsible for the variations in the effectiveness of sulfapyridine.

Since the variations in the response of different strains of type I pneumococcus to sulfapyridine do not appear to be related to such characteristics of the strains as virulence, invasiveness and antigenicity, there must be differences in other characteristics which determine the effectiveness of the drug. The work of Mellon and coworkers[17] suggests that the capacity to produce hydrogen peroxide may be a determining factor, while MacLeod's observations[18] on *"sulfapyridine-fast"* pneumococci suggest that sensitivity to the drug may depend on the manner in which carbohydrate is oxidized. These possibilities are now being investigated.

Summary. (1) Strains of type I pneumococcus varied considerably in their response to sulfapyridine. (2) These variations were not as great as the variations between types, observed previously,[2] for all the strains of type I pneumococcus were susceptible to intensive therapy. (3) Reinfection experiments indicated that there were marked variations in the antigenicity of the strains, but these differences could not be correlated with the effectiveness of sulfapyridine, and therefore were not responsible for the strain variations noted above.

---

15 Long, P. H., *J. A. M. A.*, 1939, **112**, 538.

16 Schmidt, L. H., and Hilles, C., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 111.

17 Shinn, L. E., Main, E. R., and Mellon, R. R., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 591.

18 MacLeod, C. M., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 215.