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Studies on Sulfonamide-Resistant Organisms. IV. Retention of Resistance by Pneumococci.

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It is now a well established fact that under certain conditions bacteria develop resistance to the sulfonamides. If this resistance should be a permanent characteristic and these non-susceptible organisms become widely disseminated by passage from one individual to another, sulfonamide therapy in future years may be much less successful than at present. For this reason it is important to know whether organisms which have become resistant to the sulfonamides retain this characteristic indefinitely after being removed from the influence of these drugs.

Previously reported experiments[1] showed that each of 4 strains of pneumococcus, made resistant to the sulfonamides, retained this property for at least 1 year. During this time these strains underwent approximately 200 passages through mice and an equal number in infusion broth without any contact with a sulfonamide. Since the former report, these strains have undergone similar passages for an additional 2 years. A fifth strain of pneumococcus, made resistant in another laboratory, has also been observed for a period of almost 3 years.

Experimental. Four of the strains used in this study were made resistant in this laboratory. The type I McGovern, type III CHA, and type III Wistuba strains were made resistant to sulfapyridine by serial passage through groups of mice treated with gradually increasing doses of this drug. The type II CH strain was made resistant by serial passage through broth containing increasing sulfapyridine concentrations. Details of this work have been described elsewhere.[1] The fifth strain, type I P47,* was also made resistant by serial passage through media containing sulfapyridine.[2] As originally prepared, all 5 strains were resistant both *in vivo* and *in vitro*.

These resistant strains and their sensitive parent strains were passed alternately through untreated mice and through beef infusion broth enriched with rabbit blood, either daily or every other day for approximately 3 years. At various intervals, the strains were tested for *in vivo* and *in vitro* responses to sulfapyridine.

In most of the *in vivo* tests, groups of 30 to 40 white mice (males weighing 18 to 22 g) were infected intraperitoneally. The infecting dose was 1 cc of a 10^{-6} dilution in infusion broth of a 12- to 14-hour blood broth culture obtained from the heart blood of a passage mouse. Part of the mice served as untreated controls; the remainder were treated by stomach tube with 20 mg doses of sulfapyridine. These doses, suspended in 10% gum acacia, were administered 2, 8, and 14 hours after infection and every 8 hours thereafter for 5 additional days or as long up to that time as the animals survived.

The results with 4 of the resistant strains and their sensitive parent strains are presented in Table I. Although determinations of drug sensitivity were made at approximately 6-month intervals, only the first and latest determinations are shown here, inasmuch as the results of intervening experiments were not significantly different from those described. It is evident from the data that these 4 resistant strains had essentially the same response to sulfapyridine at the end of the 3-year period as when made resistant. With reference to the corresponding parent strains, 3 showed no significant change in response to the above sulfonamide; the fourth strain (type II CH) was somewhat less sensitive at the end of 3 years passages than originally, but

[1] Schmidt, L. H., Sesler, C. L., and Dettwiler, H. A., *J. Pharm. and Exp. Therap.*, 1942, **71**, 175.

* This strain and its sensitive counterpart SV-1 were obtained from Dr. C. M. MacLeod.

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

TABLE I.
Retention of Sulfonamide Resistance by Type I McGovern, Type II CH, Type III CHA, and Type III Wistuba Strains of Pneumococcus.

Organism	Date of test	No. of mouse passages*	No. of infecting organisms	Treatment†	No. of deaths Days after infection 1	2	3	4	5	6	7-30	Avg hrs survival of mice that died	30-day survivors No.	%
Type I McGovern														
Resistant strain	5/21/40	14	1800	SP	0	22	8	0	0	0	0	47	0	0
				O	4	6	0	0	0	0	0	26	0	0
	7/28/43	605	810	SP	0	3	12	5	0	0	0	54	0	0
				O	3	7	0	0	0	0	0	26	0	0
Parent strain	6/ 7/40	22	1760	SP	0	0	0	0	0	1	2	148	27	90
				O	3	7	0	0	0	0	0	27	0	0
	7/28/43	605	560	SP	0	0	0	0	0	1	7	267	12	60
				O	2	8	0	0	0	0	0	27	0	0
Type II CH														
Resistant strain	5/21/40	12	1510	SP	0	29	1	0	0	0	0	38	0	0
				O	1	9	0	0	0	0	0	27	0	0
	9/27/43	610	290	SP	0	20	0	0	0	0	0	35	0	0
				O	3	7	0	0	0	0	0	25	0	0
Parent strain	8/18/40	60	250	SP	0	0	0	0	0	0	19	184	11	37
				O	0	10	0	0	0	0	0	30	0	0
	9/27/43	610	340	SP	0	0	0	0	0	1	19	172	0	0
				O	6	4	0	0	0	0	0	24	0	0
Type III CHA														
Resistant strain	8/ 9/40	1	300	SP	0	19	10	1	0	0	0	44	0	0
				O	7	3	0	0	0	0	0	24	0	0
	9/20/43	550	210	SP	0	19	0	0	0	0	0	36	1	5
				O	5	5	0	0	0	0	0	26	0	0
Parent strain	8/ 9/40	1	300	SP	0	0	0	0	1	0	29	186	0	0
				O	6	4	0	0	0	0	0	24	0	0
	9/20/43	550	570	SP	0	0	0	0	0	0	19	173	1	5
				O	4	6	0	0	0	0	0	27	0	0
Type III Wistuba														
Resistant strain	11/24/40	1	800	SP	0	21	9	0	0	0	0	44	0	0
				O	6	4	0	0	0	0	0	25	0	0
	9/20/43	460	500	SP	0	20	0	0	0	0	0	34	0	0
				O	9	1	0	0	0	0	0	22	0	0
Parent strain	10/28/40	‡	500	SP	0	0	0	0	0	1	15	173	4	20
				O	7	3	0	0	0	0	0	25	0	0
	9/20/43	460	550	SP	0	0	0	0	0	2	17	171	1	5
				O	4	6	0	0	0	0	0	25	0	0

* Mouse passages since preparation of the resistant strain.
† Treatment consisted of 20 mg doses of sulfapyridine, administered 2, 8 and 14 hours after infection, and every 8 hours thereafter for 5 additional days. O indicates no treatment.
‡ Wistuba parent strain not tested on 11/24/40; test of 10/28/40 nearest to above date.

was not at all like its resistant counterpart.

In contrast to the above findings, the fifth strain of pneumococcus, type I P47, lost much if not all of its resistance to sulfapyridine (Table II). There is a suggestion that this change occurred gradually, since the percentage of surviving mice increased progressively. Data on this point are not entirely conclusive, for only small numbers of mice were used in several of the intermediate experiments. It is well to note that the loss in resistance was not related to a loss of virulence; actually, the P47 strain had higher virulence at the end of 3 years than when originally received. It should also be noted that strain SV-1, the sensitive parent of P47, did not change its response to sulfapyridine during the period of observation.

The *in vitro* response of the various strains to sulfapyridine was determined at approximately the same times as the *in vivo* response. The technics of the *in vitro* tests have been detailed elsewhere.[3] The results of the tests, shown in Table III, confirm the *in vivo* find-

[3] Schmidt, L. H., Ruegsegger, J. M., Sesler, C. L., and Hamburger, M., Jr., *J. Pharm. and Exp. Therap.*, 1941, **73**, 468.

TABLE II.
Loss of Sulfonamide Resistance *in Vivo* by a Moderately Resistant Strain of Pneumococcus.

Organisms	Date of test	No. of mouse passages*	No. of infecting organisms	Treatment†	No. of mice infected	No. of deaths Days after infection 1	2	3	4	5	6	7-30	Avg hrs survival of mice that died	30-day survivors No.	%
McLeod, type I															
P47, Resistant	1/8/41	40	500	SP	30	0	0	7	9	5	0	9	113	0	0
				O	10	0	9	1	0	0	0	0	40	0	0
	1/28	60	900	SP	30	0	0	7	14	2	2	1	86	4	13
				O	10	0	9	0	0	0	0	0	41	1	10
	6/14	130	260	SP	30	0	0	0	8	3	5	12	139	2	7
				O	10	1	7	2	0	0	0	0	42	0	0
	1/15/42	225	478	SP	10	0	0	0	4	0	0	3	124	3	30
				O	5	0	5	0	0	0	0	0	28	0	0
	9/10	325	140	SP	10	0	0	0	0	0	0	0	—	10	100
				O	5	0	5	0	0	0	0	0	34	0	0
	9/27/43	470	340	SP	20	0	0	0	0	1	0	3	267	16	80
				O	10	0	10	0	0	0	0	0	32	0	0
SV-1, Parent	1/8/41	40	350	SP	30	0	0	0	0	0	0	1	—	29	97
				O	10	0	5	3	0	0	0	1	67	1	10
	1/28	60	580	SP	29	0	0	0	0	0	0	0	—	29	100
				O	10	0	9	0	0	1	0	0	50	0	0
	1/15/42	225	470	SP	10	0	0	0	0	0	0	1	—	9	90
				O	5	0	5	0	0	0	0	0	39	0	0
	10/12	325	850	SP	10	0	0	0	0	0	0	0	—	10	100
				O	5	0	5	0	0	0	0	0	39	0	0
	9/27/43	470	410	SP	20	0	0	0	0	0	0	1	—	19	95
				O	10	0	10	0	0	0	0	0	32	0	0

* Number of mouse passages after these strains were received from Dr. C. M. MacLeod.
† Treatment consisted of 20 mg doses of sulfapyridine, administered 2, 8 and 14 hours after infection, and every 8 hours thereafter for 5 additional days; O indicates no treatment.

ings reported above. An *in vivo*, the resistant McGovern, CH, CHA, and Wistuba strains showed no significant changes in response to sulfapyridine throughout the 3-year period. Likewise, the parent McGovern, CH, CHA, and Wistuba strains did not change in their reactions.

As in the *in vivo* experiments, the P47 strain lost its resistance *in vitro*. In the first experiment this strain grew in 10 mg % sulfapyridine.† Nine months later and at all times thereafter, the P47 strain was unable to grow in more than 2.5 mg % sulfapyridine. The data suggest that loss of resistance *in vitro* preceded the loss *in vivo*. The sensitive parent strain did not change in sulfapyridine sensitivity throughout the 3-year period.

Comment. The above data would seem to indicate that in some instances acquired sulfonamide resistance is a permanent characteristic, whereas in other cases it is merely a transitory property. It is noteworthy that the 4 resistant strains of pneumococcus which showed no change in sulfonamide response were highly resistant at the start of this study. On the other hand, the fifth strain, which became more responsive, was only moderately resistant at the outset. This loss of resistance by pneumococci which have acquired only moderate resistance has been observed previously in both experimental[4] and clinical[5] studies.

Previous work[6] on the development of sulfonamide resistance in pneumococci suggests

† According to the data reported by MacLeod,[2] the P47 strain grew in 1/16,000 sulfapyridine (6.2 mg %) in serum broth as compared with 1/160,000 (0.6 mg %) for the SV-1 parent strain. The differences in inhibiting concentrations found in his work and those found in this laboratory may be due to differences in experimental technics.

[4] Dettwiler, H. A., and Schmidt, L. H., *J. Biol. Chem.*, 1940, **133**, lxxxv.

[5] Hamburger, M., Jr., Schmidt, L. H., Sesler, C. L., Ruegsegger, J. M., and Grupen, E. S., *J. Inf. Dis.*, 1943, **73**, 12.

[6] Schmidt, L. H., and Sesler, C. L., *J. Pharm. and Exp. Therap.*, 1943, **77**, 165.

TABLE III.
Retention or Loss of Sulfonamide Resistance *in Vitro* by 5 Strains of Pneumococcus.

Organism	Date of test	Highest concentration of sulfapyridine permitting visible growth in 24 hr	
		Resistant strain mg %	Parent strain mg %
McGovern, type I	1/14/41	20	<5*
	9/28/43	20	5
CH, type II	1/10/41	80	<5*
	9/28/43	80	2.5
CHA, type III	1/20/41	40	5
	9/28/43	80	2.5
Wistuba, type III	1/23/41	40	5
	9/28/43	40	2.5
P47, SV-1, type I	1/ 8/41	10	<5*
	10/16/41	<2.5*	0.6
	3/ 5/42	2.5	<0.6*
	8/12/43	2.5	1.25

* Lowest sulfapyridine concentration used in this test.

that the retention or loss of resistance probably depends on the homogeneity of the organisms composing a particular resistant strain. This earlier work showed that development of a resistant strain involves a stepwise formation of pneumococci of increasing resistance, with selective propagation of the more resistant organisms in the culture and consequent elimination of the more sensitive bacteria. Thus the more resistant a strain becomes, the less likely it is to contain sensitive organisms. Such highly resistant strains as McGovern, CH, CHA, and Wistuba are probably composed solely of highly resistant organisms. On the other hand, moderately resistant strains like P47 and our earlier CHA[4] probably contained both moderately resistant and sensitive organisms. These latter strains may in time lose resistance if the particular sensitive organisms are better able to grow in the mouse or artificial culture medium than are the moderately resistant organisms.

The practical implications of these observations on the retention of sulfonamide resistance are obvious. Dissemination of highly resistant organisms would probably be a considerable hazard; dissemination of moderately resistant organisms would be a much less serious problem.

Conclusions. Strains of pneumococcus which have acquired a high degree of resistance to the sulfonamides retain that resistance for an indefinite period. Strains which have acquired only a moderate degree of resistance may lose this characteristic after removal from contact with a sulfonamide.