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Activity of Penicillin Against Strains of Pneumococci Resistant to Sulfonamide Drugs.

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We[1] reported work on penicillin in which brief mention was made of protection with penicillin against strains of pneumococci resistant to the sulfonamides *in vivo*. The work has been expanded and the present paper gives a fuller report on the subject.* At about the same time Powell and Jamieson[2] published a report on protection with penicillin and sulfapyridine against 3 strains of pneumococci Types I, II and III and their derived "sulfapyridine fast" strains. All strains except the Type I parent strain were highly resistant to sulfapyridine and all were susceptible to the action of penicillin. In the case of the Type I strain the acquisition of resistance to sulfapyridine did not change its susceptibility to penicillin.

In the work reported here 4 strains naturally resistant to the sulfonamides were used. Two Type II strains No. 617 and No. 807 were completely resistant to sulfadiazine when it was injected intraperitoneally or subcutaneously in mice or when it was administered in the food in either 0.25 or 0.5%. That is, all treated animals died, although the length of life was slightly longer in the treated than in the untreated groups. Two other strains, Type I P and Type VIII 755, were resistant but to a lesser degree than the two Type II strains since some of the treated mice survived and the difference between the survival time of treated and control mice was greater. Table I shows the relative resistance *in vivo* of these 4 strains and 2 susceptible strains. *In vitro* susceptibility of the same strains to sulfadiazine and penicillin is also shown.

There was no correlation between the resistance of the cultures to sulfadiazine *in vivo* and *in vitro*. The two strains Type II 617 and 807, most resistant *in vivo*, were more susceptible *in vitro* than the strain Type I 710 which was susceptible *in vivo*. A direct relationship seemed to exist between virulence of cultures and *in vivo* resistance to sulfadiazine. Therefore, these strains cannot be regarded as sulfonamide fast in the usually accepted sense and will be termed sulfonamide resistant. In later experiments it was shown that rapid serial passage through mice, either normal or sulfadiazine treated, rendered PnI P and PnVIII 755 more resistant to sulfadiazine apparently merely by increasing the virulence.

It was found that all strains were susceptible to the action of penicillin. In consequence more extensive experiments were carried out to determine the degree of protection achieved with penicillin as compared with sulfadiazine.

Swiss mice weighing approximately 20 g were used as the test animals. The mice were infected intraperitoneally with 0.5 cc of a 2 x 10^{-7} dilution of a 16-hr blood broth culture. This dose contained 50 to 60 organisms and, since all cultures were highly virulent for mice, represented at least 10 lethal doses. All stock cultures when not in use were kept in the frozen state. The resistant strains were passed through mice just before use and it was found that the recovered cultures killed all mice in a 10^{-7} dilution. The cultures were grown in a beef heart infusion broth containing rabbit defibrinated blood. In the course of any series of experiments it has been customary to use a culture recovered from a control mouse from the previous experiment. The penicillin-treated

[1] McKee, C. M., and Rake, G., *J. Bact.*, 1942, **43**, 645.

* The penicillin used in the present studies was supplied through the kindness of Dr. J. C. Hoogerheide of the Biological Laboratories of E. R. Squibb and Sons.

[2] Powell, H. M., and Jamieson, W. A., PROC. SOC. EXP. BIOL. AND MED., 1942, **49**, 387.

TABLE I.
Resistance of Pneumococcus Cultures to 0.5% Sulfadiazine *in vivo* and Susceptibility to Sulfadiazine and Penicillin *in vitro*.

	In vivo				*In vitro*	
		No. of mice				
Culture	Sulfadiazine	L	D	Avg length* of life	Sulfadiazine†	Penicillin†
PnII 617	0.5% in food	0	20	37 hr	4	0.3
PnII 807	"	0	20	58 "	4	0.3
PnI P	"	5	7	5 da	15.6	0.6
PnVIII 755	"	8	14	8 "	15.6	0.15
PnI 710	"	10	0		31	0.6
PnIII S	"	10	0		2	0.6

*The average length of life of control mice for the 2 Type II strains was 22 hours; for the Type I P and Type VIII strains, 24 hours; and for the 2 susceptible strains, Type I 710 and Type III S, 44 hours.

†The least amount in γ per cc which inhibited growth of approximately 800 organisms.

TABLE II.
Representative Protocols Showing Comparative Protection with Penicillin and Sulfadiazine.

Pneumococcus strain	Treatment	Doses	Florey units	No. of mice		Avg length of life in hr
				Lived	Died	
PnII 617	Penicillin i.p.	1	10	18	2	
		1	1	4	2	
		6	10	9	1	
		6	0.2	9	11	
	Sulfadiazine 0.5% in food			0	10	< 31
	Control			0	10	< 22
PnII 807	Penicillin s.c.	1	100	10	0	
		1	10	0	5	
		6	10	3	2	
	Sulfadiazine 0.25% in food			0	10	< 48
	Control			0	10	< 24
PnI P	Penicillin i.p.	1	10	20	0	
		1	1	19	1	
	Penicillin s.c.	1	100	20	0	
		1	10	4	16	
	Sulfadiazine 0.25% in food			0	10	< 48
	" 0.5% " "			0	10	< 92.6
	Control			0	10	< 24
PnVIII 755	Penicillin i.p.	1	100	10	0	
		1	10	10	0	
		1	1	9	1	
		6	10	10	1	
		6	1	10	1	
	Penicillin s.c.	1	100	10	0	
	Sulfadiazine 0.25% in food			0	10	< 54.4
	" 0.5% " "			0	10	<113.8
	Control			0	10	< 25.6

mice were injected either intraperitoneally or subcutaneously immediately after infection. In some cases only one injection was given, at other times three injections a day for two days were made. Injections were spaced about 4 hr apart at 9 A.M., 1 and 5 P.M. No injections were made during the night. A sodium salt of penicillin was used, the potency of which was 100 Florey units per mg. When administered intraperitoneally as little as one Florey unit or 0.01 mg protected a large number of the mice so treated. When treatment was given subcutaneously, *i.e.* at a distance from the site of infection, more penicillin was necessary for protection. Ten Florey units gave some protection and 100 Florey units complete protection. Sulfadiazine was administered in the food in either

TABLE III.

Summary of Comparative Protection with Penicillin and Sulfadiazine.

Pneumococcus strain	Dilution of culture	Treatment				No. of mice	
		Material	Route	Dose	Florey units	Lived	Died
PnII 617	10-7	P*	i.p.	1	10	24	2
	,,	,,	,,	1	1	13	3
	,,	,,	,,	6	10	39	2
	,,	,,	,,	6	1	6	0
	,,	,,	,,	6	0.2	15	11
	10-6	,,	,,	1	1	3	7
	10-5	,,	,,	1	100	5	5
	,,	,,	,,	6	10	10	3
	10-4	,,	,,	6	10	3	3
	10-7	,,	s.c.	1	100	16	0
	,,	,,	,,	1	10	1	4
	,,	,,	,,	6	10	1	4
	10-5	,,	,,	1	100	1	9
	,,	,,	,,	1	50	0	10
	10-7	SD*		.25%		0	15
	,,	,,		.5%		0	46
	,,	C*				0	64
PnII 807	10-7	P	i.p.	1	10	22	3
	,,	,,	,,	1	1	18	7
	,,	,,	,,	6	10	32	0
	,,	,,	,,	6	1	11	4
	,,	,,	,,	6	0.2	1	4
	10-6	,,	,,	1	100	10	1
	,,	,,	,,	6	10	5	1
	10-5	,,	,,	6	10	0	10
	10-7	,,	s.c.	1	100	16	0
	,,	,,	,,	1	10	0	5
	,,	,,	,,	6	10	3	2
	10-6	,,	,,	1	100	9	1
	,,	,,	,,	1	50	9	1
	10-7	SD		.25%		0	15
	,,	,,		.5%		0	46
	,,	C				0	64
PnIP	10-7	P	i.p.	1	10	51	1
	,,	,,	,,	1	1	46	6
	10-6	,,	,,	6	10	20	0
	10-7	,,	s.c.	1	100	52	0
	,,	,,	,,	1	10	21	31
	,,	SD		.25%		0	20
	,,	,,		.5%		0	32
	,,	C				1	31
PnVIII 755	10-7	P	i.p.	1	10	20	0
	,,	,,	,,	1	1	19	1
	,,	,,	,,	6	10	20	0
	,,	,,	,,	6	1	20	0
	10-6	,,	,,	6	10	20	0
	10-7	,,	s.c.	1	100	20	0
	,,	SD		.25%		0	20
	,,	,,		.5%		0	20
	,,	C				0	20

* P = Penicillin, 100 Florey units per mg.
SD = Sulfadiazine in food.
C = Control.

0.25 or 0.5%. All mice were put on the drug-containing diet 2 days before infection. Those mice receiving 0.5% drug in the food lived a little longer than those receiving 0.25%. Typical protocols of experiments with the 4 strains of pneumococci are given in Table II. In all cases the infecting dose was .5 cc of a 2 x 10^{-7} dilution given intraperitoneally. Since penicillin is so quickly excreted, repeated treatments at short intervals are usually given. It is of interest, therefore, as shown in the above protocols, that we obtained excellent protection with a single dose. In the case of PnII 617 it will be seen that a single dose of 10 Florey units gave as good protection as repeated doses. This suggests that penicillin exerts bactericidal as well as bacteriostatic action.

Pooled results of all experiments with PnII 617, and PnII 807 and of those experiments with PnI P and PnVIII 755 made after the strains had been rendered more virulent by mouse passage, are given in Table III. These results include infection with larger numbers of organisms, and in such cases differences in susceptibility of strains to penicillin become apparent. Penicillin afforded some protection against infection with PnII 617 even when the dose of organisms was increased 1000 fold but with PnII 807 it protected only against a ten-fold increase, not against 100 fold. Complete protection against PnI P and PnVIII 755 was achieved with 10 times the usual dose of organisms, the largest number tried.

As mentioned above, when an attempt was made to increase the resistance to sulfadiazine of PnI P and PnVIII 755 it was found that rapid serial passage through either normal or sulfadiazine-treated mice so increased the virulence that all mice treated with sulfadiazine died. Both strains were equally susceptible to the action of penicillin and equally virulent for the control mice. This suggests the possibility that in some other reported cases apparent drug fixation acquired by animal passage may be due, at least in part, to increased virulence.

An effort was made to produce penicillin resistant strains. Cultures of PnII 617 and 807 recovered from penicillin treated mice were grown for 8 transfers in broth containing the greatest amount of penicillin which would permit growth. When tested *in vitro* the penicillin passage strains proved to be twice as resistant as the unpassed strains to the action of penicillin. However, when tested in mice no difference in resistance was observed. Abraham, Chain *et al.*[3] were able to adapt a *Staphylococcus aureus* culture to penicillin by growing the culture in penicillin-containing broth so that after a few daily subcultures the organism grew in twice the previously inhibitory concentration. In 9 weeks, with subcultures every few days an adaptation to a 30-fold concentration was reached and in a further 7 weeks the adapted strain grew in a concentration 1000 times greater than that which inhibited the original culture. No *in vivo* tests were made with the penicillin resistant strain. Passage with penicillin either *in vitro* or *in vivo* over a longer period of time might render our pneumococcus cultures more resistant.

Summary. Four strains of pneumococci naturally resistant to sulfonamide action have been shown by mouse protection tests to be readily susceptible to the action of penicillin. When infection and treatment were both given intraperitoneally a single dose of one Florey unit protected 84%, and 10 Florey units protected 95% of the treated mice; 6 doses of one Florey unit per dose protected 90% and 6 doses of 10 Florey units protected 99% of the treated mice. When infection was given intraperitoneally and treatment subcutaneously a single dose of 10 Florey units protected 35%, and 100 Florey units protected 100% of the treated mice.

Cultures of PnII 617 and 807 after growth in penicillin broth for a short time were resistant to twice the previously inhibitory concentration. No difference in resistance could be shown by mouse protection test.

[3] Abraham, E. P., Chain, E., Fletcher, C. M., Gardner, A. D., Heatley, N. G., Jennings, M. A., and Florey, H. W., *Lancet*, 1941, **2**, 177.