Effect of Prior Immunization on Bactericidal Action of Penicillin *in vivo*. (20066)

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The present experiments attempt to assess the degree to which the bactericidal action of penicillin may be modified by the presence of antibodies. It had previously been shown in an early group A streptococcal infection of non-immune mice that cure was achieved only if the antibiotic effected virtual sterilization (1-3). Even when more than 99.99% of the organisms had been killed under the impact of the drug, the few organisms still surviving after the penicillin had fallen to ineffective levels regularly recovered and remultiplied to cause a fatal infection. As will be here shown, prior immunization with the homologous organism had no effect either on the rate at which penicillin killed the bacteria *in vivo*, or on the duration of its action. The outcome of treatment was, however, strikingly modified. In these partially immune mice, a small fraction of the normally curative dose of penicillin sufficed to reduce the bacterial population below the number which could be handled by the host defense mechanisms. Under these circumstances, the bacteria surviving the direct action of penicillin did not remultiply, but slowly disappeared, and the animals recovered.

Experimental. Exp. 1. One hundred white mice weighing 18 to 22 g were a) inoculated intraperitoneally with 4×10^6 group A streptococci (C-203 strain of *Streptococcus pyogenes*), and 2 hours later were treated intramuscularly in the left hind leg with 200 mg/kg of aqueous sodium penicillin G. This treatment was repeated 7 hours after inoculation. The virulence of the strain had been maintained by passage through mice twice weekly for a period of 3 years. b) The same sequence of inoculation and treatment was repeated in all the animals after 12 days. c) Eight days after the second immunizing inoculation the animals were inoculated into the right leg muscle with 4×10^6 bacteria; and in 50 of the mice, penicillin at a dosage of 12.5 mg/kg was injected 2 hours later. Groups of 6-8 mice were sacrificed at varying intervals after treatment in order to determine the number of organisms surviving in the inoculated leg muscle, as previously described (1-3).

1) *The degree of immunization* afforded by the 2 immunizing injections is shown in Table I. In this experiment, the LD_{50} was on the order of 4×10^6 organisms in the immunized animals, and approximately 10 in normal controls simultaneously inoculated.

2) *The rate of bacterial multiplication in normal and immunized mice.* When the immunized mice were inoculated with 4×10^6 organisms, the bacteria initially grew out at essentially the same rate as in normal animals (top half of Table II). After 8 hours, however, the average number of viable organisms was significantly lower in the immunized animals; and the difference between the 2 groups became progressively more pronounced. The eventual mortality in the immunized mice receiving this inoculum was 8/20.

3) *The effect of treatment* with penicillin

TABLE I. Effect of Prior Immunization with Group A Streptococci on LD_{50} Inoculum (Exp. 1).

Mice		Mortality of mice receiving indicated number of organisms intramusc.			Approx. LD_{50}
Normal	Inoculum	10^1	10^2	10^3	10
	Mortality	10/20	19/20	18/20	
Immunized	Inoculum	4×10^5	4×10^6	4×10^7	4×10^6
	Mortality	1/15	8/20	15/20	

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TABLE II. Rate at Which Group A Streptococcus Multiply in Normal and Previously Immunized Mice.*

Exp.	Mice	Millions of organisms in inoculated muscle at indicated time (hr) after inoculation												Mortality after 28 days, untreat. mice
		0†	2		4		8		24		48		72	
1	Normal	3	25	19			313	157	346	154	154	99	576	10	20/20
		2.8	23	18			310	157	198	77	128	58	115		
		2.7	21	15			224	131	160	58	121	35	42	(3 mice
		2.7	19	11			166	112	160	15	99	22	30	dead)
		2.6												
	Immunized	2.5	24	12			64	7.1	64	7.5	27	6.2	12.8	.0037	8/20
		2	23	9.5			51	6.4	35	3	26	.032	4.9	0	
		1.9	20	9.4			51	6.2	27	2.3	7.5	.024	.028	0	
			15	6			43	2.8	18	.0016	6.9	.024	.0073	0	
2	Normal		62	41	278	77	404	222							20/20
		6.5	51	23	272	32	403	179							
		5.1	46		195		323								
		3.8	46		157		304								
		3.2													
	Immunized	3	72	51	200	99	426	102							20/20
		3	62	23	179	84	336	102							
			56	20	170		266	102							
			51	13	141		144								

* Mice were inoculated intramuscularly with 4×10^6 organisms (by microscopic count). In Exp. 1, the plate count on the inoculum was 2.3×10^6, and in Exp. 2, 4.5×10^6 colonies.

† Immediately after inoculation there was no significant difference in the number of viable bacteria in the normal and immunized mice, and at this time period the two groups have been combined in the table.

TABLE III. Effect of Treatment with Penicillin on an Intramuscular Focus of Group A Streptococci in Normal and Partially Immune Mice.

Exp.	Penicillin dosage, mg/kg			Thousands of bacteria in inoculated muscle at indicated time after treatment (hr) 0 (×1000)	1½	3	6	9	12	24	48	Mortality in penicillin treated mice
1	12.5	Normal*	Range of observations†	16–51	62–92	4.2–148	11–42	124–844	1200–13100			20/20
			Mean‡	27.8	74	16.2	20	374	3460			
			S.E.(×/÷)§	1.17	1.05	1.54	1.18	1.31	1.34			
		Immunized*	Range†	20–32	8.6–34	2.9–8.8	.3–78	4 mice=0 4 mice=.05–4.7	7 mice=0 1 mouse=.05	6 mice=0 2 mice= 5.5–1000	0	0/20
			Mean‡	25.3	13.5	5.35	1.29	—	—	—	—	
			S.E.(×/÷)§	1.06	1.02	1.15	2.64					
2	3.2	Normal	Range†	7.7–23	14–52	5.2–524	6.7–322	142–7700	1280–29000	720–400000		20/20
			Mean‡	12.8	23.6	35	29.4	1120	7760	494000		
			S.E.(×/÷)§	1.17	1.24	2.1	1.86	1.91	1.72	2.76		
		Immunized	Range†		.65–118	5.2–32	.3–28	.08–32	.1–24000	2 mice=0 6 mice= 5.4–1360	2 mice died 1 mouse=0 5 mice= .05–19600	13/20
			Mean‡	12.8	19.9	12.1	1.95	2.22	4.96			
			S.E.(×/÷)§	1.17	1.9	1.25	1.74	2.1	4.53			

* In Exp. 1, normal and immunized mice were inoculated and treated on different days. In Exp. 2, the same bacterial suspension was used in both groups, and the same zero time values apply to both (*cf.* footnote †, Table II).

† 6-8 mice in each experimental group. ‡ Geometric mean.

§ S.E. (stand. error) is the antilog of the stand. error of the mean logarithm. The stand. error of the mean logarithm was estimated as $\frac{\text{range of log counts}}{\text{No. of animals}}$, after Mantel (4).

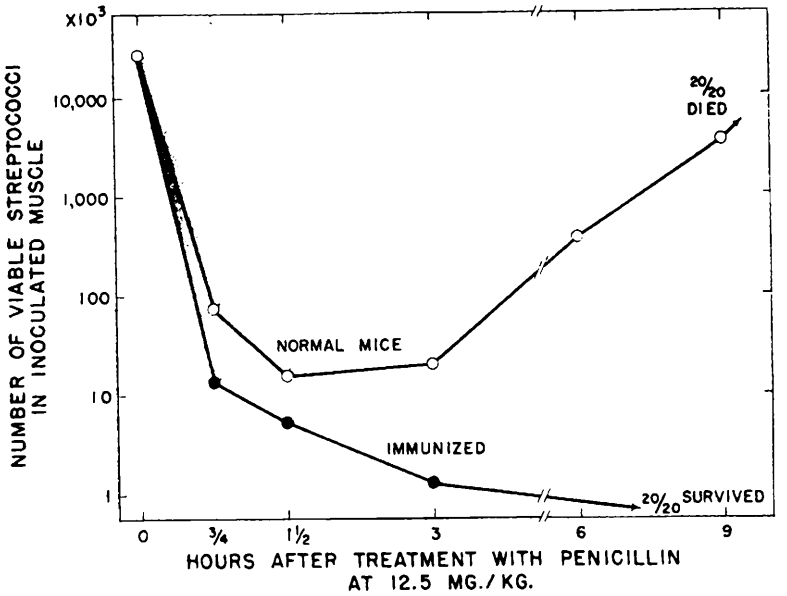

FIG. 1. The bactericidal action of a single dose of sodium penicillin G (12.5 mg/kg) in normal and immunized mice infected with group A streptococci (from data of Table III). The cross-hatched portion of the curve indicates the avg time for which the serum concentration of penicillin remains in excess of .05 μg/ml after this dosage(3).

in the immunized mice and in normal controls is shown in Table III and Fig. 1. The rapid bactericidal action of penicillin proceeded at the same rate and for essentially the same period in both groups. The single intramuscular dosage of 12.5 mg kg of aqueous sodium penicillin used in this experiment provides a serum concentration in excess of 0.05 μg kg for approximately 1.7 hours and in excess of 0.02 μg/kg for approximately 2.0 hours(3). These concentrations have been estimated to provide the maximally and minimally effective concentrations at the focus of infection(3); and the bactericidal effect continued for approximately the time that the serum concentration remained in excess of these levels. However, once the penicillin had fallen to ineffective levels, the course of infection deviated markedly in the 2 groups of mice. In the normal controls, all the animals treated at this dosage level died. The number of viable bacteria surviving this subcurative dose of 12.5 mg kg remained essentially constant in number for approximately 4-6 hours, and then regularly resumed remultiplication to cause a fatal infection. In the immunized mice, however, the surviving bacteria had been reduced to a mean level of 13,500, much less than the number of organisms necessary to induce a fatal infection (Table I). As indicated in Fig. 1 and Table III, the organisms surviving the action of penicillin gradually disappeared over the following 24-48 hours, and all of 20 treated animals survived.

Exp. 2. Qualitatively similar results were obtained in a second experiment. In this experiment, 20 days elapsed between the 2 immunizing injections; and 12 days elapsed between the second immunizing injection and the final test of penicillin action. Either because of this change, or because of a difference in the condition of the immunizing culture, the immune response was much less marked in this second experiment. An inoculum of 4×10^4 bacteria killed every one of 20 animals tested, as compared with an LD_{50} of 4×10^6 in the first experiment; smaller doses were unfortunately not tried. Despite the less pronounced immunity, the results obtained were qualitatively the same as in the first experiment. For the first 8 hours, bacteria inoculated into these immunized mice grew out at exactly the same rate as in normal animals simultaneously inoculated (Table II). When

the infected animals were treated with penicillin (this time at a dosage of only 3.2 mg/kg), the bactericidal effect proceeded at the same rate and for the same period of time in the normal and in the immunized mice (bottom half of Table III). This dose of 3.2 mg/kg provides levels of 0.05 and 0.02 mg/kg for 1.1 and 1.4 hours, respectively; and the bactericidal effect of penicillin continued for approximately this period. At this point, 1½ hours after treatment, the number of organisms had been reduced to a mean level of 20,000-25,000 in both groups of animals. Once again, in all the normal controls, bacteria which survived the action of penicillin eventually recovered, and rapidly grew out to cause a regularly fatal infection. In the immunized animals, however, 7 of 20 mice were cured by the single dose of 3.2 mg/kg. (This was approximately 1/35th the dose normally necessary to cure half the animals.) The bacteria surviving the action of penicillin were gradually disposed of by the host defense mechanisms, the muscle became sterile, and the animals survived (Table III). In 13/20 of these animals, however, because of the less pronounced immunity effected in this experiment. some of the surviving organisms recovered and remultiplied to cause a fatal infection. The varying course of the infection in the animals which died and those which survived is evident in the bottom half of Table III.

Summary and discussion. In the group A streptococcal infection of mice here studied, and under the conditions of the present experiments. the presence of a significant degree of immunity had no effect on the direct bactericidal action of penicillin. This proceeded at the same rate in the immunized animals as in control mice simultaneously inoculated, and continued for the same period of time. The difference in the 2 groups lay in the course of the infection after penicillin had fallen to ineffective levels. In non-immune mice, the surviving bacteria regularly grew out to cause a fatal infection. In the partially immunized animals, however, a small fraction of the normally curative dose of penicillin sufficed to reduce the bacterial population to levels which could be handled by the host defenses. Those surviving bacteria gradually disappeared in the course of 24-48 hours, after penicillin itself was no longer operative, and the animals survived. Under the conditions of this experiment, the host defenses and the bactericidal action of penicillin proceeded independently of each other, but were mutually supplementary in effecting cure in the partially immunized animals.

The choice of a group A streptococcal infection was perhaps unfortunate. The resistance to reinfection in the immunized animals probably reflects enhanced phagocytosis; and in the present experiments, there was no pronounced cellular infiltration at the focus of infection at the time of treatment with penicillin. It would be of interest to carry out similar studies under conditions closer to those usually operative in the natural infection of man, with the cellular host defenses already mobilized at the focus of infection. It would be of interest also to determine, in infections caused by organisms susceptible to the bactericidal action of antibodies and complement, whether the presence of such antibodies modifies the therapeutic action of penicillin and other antibiotics.

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