Experimental Bacterial Endocarditis in Altitude Rats. III. Effect of Age of Infection on Response to Penicillin. (19802)

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In previous papers(1-3), it was shown that severe cardiac damage could be induced in rats by 4-hour daily exposures in a low-pressure chamber to a simulated altitude of 25,000 feet, and that bacterial endocarditis and renal lesions could be readily produced in such rats by the intravenous injection of bacteria. In this paper, we are reporting on the response of such experimentally-induced endocarditis and renal lesions to penicillin therapy, and the effect of the age of the infection on the therapeutic response. The methods used in exposing the animals to altitude, in inoculating the bacteria, and in preparing the histologic sections, were essentially similar to those described in the previous reports(1-4).

Methods. In the first experiment, 64 young adult white male Holtzmann rats received 70 successive daily exposures to 25,000 feet simulated altitude. The rats, weighing about 250 grams each, were then inoculated intravenously with 0.5 cc of a 6-hour culture of *Streptococcus faecalis* in dextrose blood broth, with a microscopic count of 1 billion bacteria per cc. Treatment was begun 20 hours later. The animals, except those used as controls, were given 200 mg/kg of sodium penicillin G in aqueous solution 6 times daily at 3, 9, and 12 a.m., 3, 6, and 11 p.m. At the intervals indicated in Table I, rats were killed for bacteriologic and histopathologic studies. For the bacteriologic studies, the hearts and kidneys were removed aseptically, emulsified in 50 cc of 1% dextrose blood broth in a Waring blendor for 1½ minutes, and 1 cc of the emulsion was subcultured in dextrose blood

TABLE I. Exp. 1. Effect of Sodium Penicillin G (200 mg/kg 6 Times Daily Intramusc.) on Bacterial Counts* in Hearts and Kidneys of Rats Inoculated 20 Hr Previously with a Single Intrav. Inj. of *Streptococcus faecalis*.

		Days after inoculation	1	2	3	5
		Days after treatment	0	1	2	4
Heart	Untreated controls		2160	606	182400	1760
			108	460	10600	1680
			78	192	120	0
			22			
			6			
			4.7			
	Treatment instituted 20 hr after inoc.			.35	1096	122
				.2	0	.05
				.1	0	.05
				.05	0	0
				0	0	0
Kidney	Untreated controls		16300	131200	141000	550000
			16100	9600	83000	277000
			16600	4800	38000	237000
			10400			
			7300			
			1300			
	Treatment instituted 20 hr after inoc.			434	26	
				62	10.3	508
				20	9.4	1.7
				14	4.6	.5
				0	2.4	.45
						0

* Numbers in the table are total numbers of streptococci in thousands. 0 = <20 organisms in the organ emulsion.

TABLE II. Exp. 2. Effect of Intramusc. Treatment with Sodium Penicillin G* on Bacterial Counts† in Hearts of Rats Inoculated with *S. faecalis*.

Days after inoc.	½	1½	2½	4½	5½	6½	8½	10½
Untreated controls	246	430	43000	48000		146000	7500	
	72	320	252	3200		140000	1.1	
	51	210	190	128		2100	.2	
	30	13	4.1	120		.7		
	28	2.9	.7	78		0		
	13			.7				
	6.8							
Treatment instituted early, 12 hr after inoc.		12	106	.57		.17		
		2.9	.033	.47		.17		
	Penicillin	3.1	.033	0		0		
	begun	2.2	0	0		0		
		1	0	0		0		
		.5	0	0		0		
Treatment instituted late, 4½ days after inoc.					67000	90000	.033	1950
					61000	25000	0	.033
			Penicillin begun		28.7	.67	0	.03
					1.5	.67	0	0
						<.6	0	
							0	

* Treatment at 200 mg/kg 4 times daily plus 20 mg/kg "Benemid" twice daily.
† Numbers in table represent total bacterial count in thousands. 0 = <20 organisms in the total heart emulsion.

TABLE III. Exp. 2. Effect of Intramuscular Treatment with Sodium Penicillin G* on Bacterial Counts† in Kidneys of Rats Inoculated with *S. faecalis*.

Days after inoc.	½	1½	2½	4½	5½	6½	8½	10½
	× 1000							
Untreated controls	2.85	1430	406	2050		2600	4142	
	1.60	66	137	1500		2400	1238	
	.91	53	32	1370		2050	1070	
	.85	33	5.7	642		1370		
	.67	11	4.3	534		934		
	.61			192				
	.35							
Treatment instituted early, 12 hr after inoc.		750	400	1200		3000000		
		501	47	1.9		86.4		
	Penicillin	320	42	.95		40.5		
	begun	124	31	.77		1.23		
		64	3.7	.39		0		
		33	1.7	0		0		
					× 1000			
Treatment instituted late, 4½ days after inoc.					693	2600	255	107
					420	2396	173	107
			Penicillin begun		353	1505	4	55
					73	453	0	1.9
						406	0	
							0	

* Treatment at 200 mg/kg 4 times daily plus 20 mg/kg "Benemid" twice daily.
† Numbers in table represent total bacterial count in thousands. 0 = <20 organisms in the total kidney emulsion.
It is to be noted that the numbers in the top and bottom sections are to be multiplied by 1000 to give the count in thousands.

agar. In a second experiment, 183 young adult white male Holtzmann rats received 30 successive daily exposures to altitude and then were inoculated intravenously with 0.5 cc of a 4-hour streptococcal culture. The inoculum contained 1150 x 10^6 organisms/cc by microscopic count, and 840 x 10^6 by plate count. The animals were then divided into 3

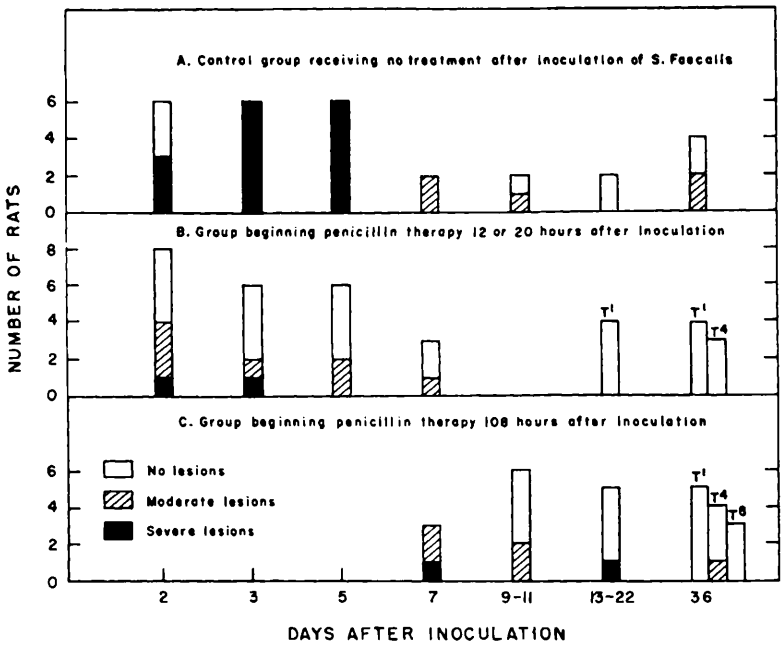

FIG. 1. Effect of penicillin therapy on development of cardiac lesions in altitude rats inoculated with *Streptococcus faecalis*. All rats in groups (B) and (C) were treated until end of specified period except those in columns T^1, T^4, and T^8, which were treated for only 1, 4, and 8 days respectively. In columns marked 13-22, rats in groups (A) and (C) were killed at 13 to 16 days after inoculation and rats in group (B) at 22 days. No spontaneous deaths are included.

groups. Two groups were treated intramuscularly 4 times daily (9:00 a.m., 1:00 p.m., 5:00 p.m., 11:00 p.m.) with sodium penicillin G at 200 mg/kg and twice daily with "Benemid"* at 20 mg/kg. Treatment of the first group was begun at 12 hours and of the second group at 4½ days after inoculation. The third group served as an untreated control. Bacteriologic studies were made as described in the first experiment. In the control, untreated animals the bacterial counts in the blood at autopsy (1 cc samples) varied from 0 to 244 per cc, numbers which were insignificant in relation to those found in the heart and kidney. In all but one of the treated animals, the bacterial count in the blood at autopsy was less than 23 per cc; and in more than two-thirds of the treated animals, duplicate blood cultures on 1 cc samples were negative, including animals with massive bacterial involvement of the heart and kidney.

Results. a) *Bacteriologic studies.* As shown in Tables I, II, and III, in both the heart and kidney, early treatment, begun either 12 or 20 hours after inoculation usually effected a significant and fairly rapid reduction in the bacterial count, as contrasted with the rapid and progressive increase in the control untreated animals. However, when treatment was delayed until 4½ days after inoculation, the effect of penicillin on the bacterial count in the kidney was not apparent until the 8th day, 4 days after the beginning of treatment. The effect of delayed treatment on the bacterial count in the heart was obscured by the variability of the results obtained in both the control and treated animals.

b) *Pathology.* The histopathologic findings in the 2 experiments are summarized in Fig. 1 and 2. The lesions were graded severe (black in the columns) only if bacteria were noted in the microscopic sections. In the heart, streptococci occurred in large masses in the valvular vegetations and in the kidneys they were usually found in necrotic lesions in the renal papillae. In the kidneys there were also scattered inflammatory foci of variable size, in which were tubules contain-

* Furnished by Sharp and Dohme.

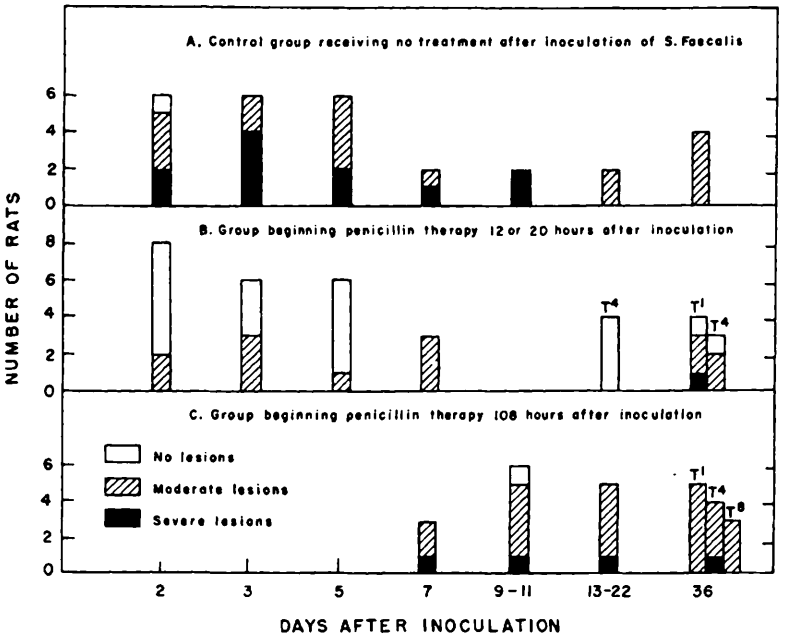

FIG. 2. Effect of penicillin therapy on development of renal lesions in altitude rats inoculated with *Streptococcus faecalis*. All rats in groups (B) and (C) were treated until end of specified period except those in columns T^1, T^4, and T^8, which were treated for only 1, 4, and 8 days respectively. In columns marked 13-22, rats in groups (A) and (C) were killed at 13 to 16 days after inoculation and rats in group (B) at 22 days. No spontaneous deaths are included.

ing a purulent exudate occasionally admixed with bacteria. Lesions were graded as moderate if there were inflammatory changes without evident bacteria. These represent mild, early developing, or late regressing lesions. In the heart, regression or healing of severe lesions was suggested by the occurrence of large organizing vegetations, in which there were no visible bacteria and relatively few polymorphonuclear leucocytes. In the kidney, regression was evidenced by depressed scars infiltrated by lymphoid cells, with a purulent exudate in a relatively small number of tubules. Calcification was sometimes noted in a few scattered tubules, and occasionally there was apparent sloughing into the pelvis of the necrotic portion of a renal papilla.

The 12-hour and 20-hour treatment groups have been combined in the figures since in both, the early treatment appeared to have been effective in suppressing the development of serious cardiac or renal lesions during treatment. This agrees with the rapid disappearance of bacteria from these organs (Tables I, II, III). Treatment was perhaps more effective in the 12-hour group, since only 1 of 11 rats in this group examined immediately after treatment for 1 to 4 days showed lesions, consisting of a sterile vegetation and a focal leucocytic infiltration of the mitral valve. In contrast, 7 of 9 similarly treated rats in the 20-hour group had moderate to severe valvular lesions and 6 had moderate suppurative renal lesions. None of the 11 rats treated 12 hours after the inoculation and killed for histologic study after penicillin treatment for 1 to 4 days had significant renal lesions. However, 4 to 5 weeks later, 5 of 7 animals in this group (Fig. 2, group B) had regressing suppurative renal lesions as described above. These findings are consistent with the fact that such early treatment usually suppressed the multiplication of bacteria, but had sterilized the kidneys in only 3 of 12 rats examined immediately after treatment for 4 to 6 days. It is evident, therefore, that treatment for 4 to 6 days may temporarily suppress the kidney infection, but often does not prevent subsequent bacterial multiplication and the development of lesions in this group.

In the animals in which treatment was not begun until 4½ days after inoculation, treatment had little effect on the renal lesions, which by that time ranged from moderate to severe. This agrees with the fact that the bacterial count in the kidneys also was not immediately affected in this group.

The effect of delayed treatment on the cardiac lesions is difficult to evaluate because of the small number of controls, and because the incidence and severity of cardiac lesions were relatively low in animals which survived without treatment (Fig. 1). The lesions tended to be more severe in animals that died than in those that were killed for study at corresponding periods. These spontaneous deaths have not been included in the tables or figures.

c) *Mortality.* The cumulative mortality† in untreated rats during the first 4½ days following inoculation was 8%. In the following 5 weeks, the cumulative mortality in the surviving untreated rats (controls) was 55%. Over the same period, in rats beginning treatment 4½ days after inoculation, the cumulative mortality was 17%. In animals treated 12 hours after inoculation, the cumulative mortality was 2%, one of 51 rats dying 8 hours after the beginning of treatment.

(d) *Discussion.* The contrasting effects of early and late treatment on the bacterial count, on the histopathologic findings in the heart and kidney, and on the mortality, agree qualitatively with the results previously reported in a group A streptococcal infection in mice(5). Both infections were easily controlled by early treatment with penicillin, but were relatively refractory to treatment instituted later, after there had been marked bacterial proliferation. These findings may have a bearing on the known refractoriness of bacterial endocarditis to treatment with penicillin. Possible explanations have been discussed in the previous paper(5).

Summary and conclusions. Rats were exposed daily to simulated high altitude and then inoculated intravenously with a young broth culture of *Streptococcus faecalis.* (a) Untreated rats developed a vegetative bacterial endocarditis and renal abscesses and necrosis. (b) Treatment with penicillin instituted within 12 or 20 hours after inoculation was highly effective in reducing the mortality, and in aborting or preventing the development of cardiac lesions. In the kidney, such early treatment effected an immediate marked reduction in the average bacterial count and in the incidence and severity of lesions. However, 4-5 weeks after the cessation of treatment renal lesions were noted in 5 of 7 animals, presumably caused by surviving bacteria. (c) When treatment was delayed until 4½ days after inoculation, permitting extensive bacterial proliferation and the development of pathological changes, penicillin therapy had a much less favorable effect on mortality, bacterial counts, and the regression of lesions.

† Calculated for each experimental group by a modified life table technic. Mortality rate was not computed for rats treated 20 hours after inoculation since nearly all were killed within 5 days.

1. Highman, B., and Altland, P. D., *Arch. Path.*, 1949, v48, 503.
2. ———, PROC. SOC. EXP. BIOL. AND MED., 1950, v75, 573.
3. ———, PROC. SOC. EXP. BIOL. AND MED., 1951, v78, 590.
4. Altland, P. D., *J. Exp. Zool.*, 1949, v110, 1.
5. Eagle, H., *Am. J. Med.*, 1952, v13, 389.

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