

IV. Effect of Subtilin on the Course of Experimental Anthrax Infections in Guinea Pigs.*

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In previous communications^{1,2} it was reported that subtilin showed an extremely low toxicity index to living embryonic chick heart tissue fragments cultivated *in vitro* and that it exerted a powerful *in vivo* action on the course of experimental pneumococcus Type III infections in mice. Animals treated with subtilin 9 hours after being injected with the organism were quickly cured of the infection. The antibiotic did not exhibit any apparent toxic reaction in the mice.

Subtilin is effective chiefly against Gram-positive bacteria *in vitro*.³ A few Gram-negative organisms are equally as susceptible. However, the great majority of Gram-negative organisms are not susceptible unless considerably higher concentrations are used. *Bacillus anthracis* is Gram-positive and easily destroyed *in vitro*.

In the present communication, results are given on the effectiveness of subtilin on the course of experimental anthrax infections in guinea pigs.

Experimental. Two guinea pigs weighing approximately 500 g each were injected intraperitoneally with 0.1 cc of a 24-hour nutrient broth culture of *Bacillus anthracis*. One animal was not treated but served as the control; the other was treated immediately with 3 cc of a solution containing 2 mg subtilin per cc (total of 60 units¹) and at stated intervals thereafter. The subtilin was dissolved in a 5% solution of glucose and all injections were made intraperitoneally. The

TABLE I.
Treatment of Anthrax in Guinea Pigs.

Date	Time	Control animal	Treated animal
2-14-46	12:30 PM	0.1 cc culture	0.1 cc culture
	" "	—	3 cc subtilin
	3:30 "	—	3 "
	6:00 "	—	3 "
	11:00 "	—	3 "
15	2:00 AM	—	3 "
	4:30 "	—	3 "
	6:30 "	—	3 "
	9:30 "	—	3 "
	12:30 PM	—	3 "
	3:30 "	—	3 "
	9:30 "	—	3 "
16	1:30 AM	—	3 "
	4:30 "	—	3 "
	9:30 "	—	3 "
	3:00 PM	—	3 "
	10:00 "	Animal died	3 "
17	10:30 AM	—	3 "
	8:00 PM	—	3 "
18	10:30 AM	—	3 "
19	3:30 PM	—	Treatments discontinued
28	12:00 M	—	Animal died

treatments were discontinued after the 4th day and the animal kept under observation. The schedule of treatments and results are recorded in Table I.

It may be seen that the control animal (untreated) died in less than 72 hours. The treated animal was given subtilin for 4 days, then observed for an additional 10 days (total 14 days) when death occurred.

Since the culture of *Bacillus anthracis* contained many spores, it is believed that these must germinate into vegetative cells before they can be destroyed by the subtilin. It would appear that some spores did not germinate until after treatment was discontinued.

A second experiment was performed in which 4 guinea pigs were treated instead of only one, and the treatments continued for a longer period of time. The animals were each inoculated with 0.1 cc of a 24-hour nutrient broth culture of the organism, and varying periods of time were allowed to elapse

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¹ Salle, A. J., and Jann, G. J., *PROC. SOC. EXP. BIOL. AND MED.*, 1946, **61**, 23.

² Salle, A. J., and Jann, G. J., *PROC. SOC. EXP. BIOL. AND MED.*, 1946, **62**, 40.

³ Salle, A. J., and Jann, G. J., *PROC. SOC. EXP. BIOL. AND MED.*, 1945, **60**, 60.

TABLE II.
 Treatment of Anthrax in Guinea Pigs.

Date	Time	Animals inoculated intraperitoneally with 0.1 cc culture				
		Control not treated	Treated immediately cc subtilin	Treated after 3 hrs cc subtilin	Treated after 6 hrs cc subtilin	Treated after 9 hrs cc subtilin
3-5	2:00 PM	—	3			
	5:00 "	—	3	3		
	8:00 "	—	3	3	3	
	11:00 "	—	3	3	3	3
6	2:00 AM	—	3	3	3	3
	5:00 "	—	3	3	3	3
	8:00 "	—	3	3	3	3
	11:00 "	—	3	3	3	3
	2:00 PM	—	3	3	3	3
	5:00 "	—	3	3	3	3
	8:00 "	—	3	3	3	3
	11:00 "	—	3	3	3	3
7	2:00 AM	—	3	3	3	3
	5:00 "	—	3	3	3	3
	9:30 "	—	5	5	5	5
	1:00 PM	—	5	5	5	5
	10:00 "	—	5	5	5	5
8	10:00 AM	—	5	5	5	5
	4:00 PM	—	5	5	5	5
	7:00 "	Dead	5	5	5	5
9	9:00 AM		5	5	5	5
	5:00 PM		5	5	5	5
10	9:30 AM		4	4	4	4
	4:30 PM		5	5	5	5
	9:30 "		5	5	5	5
11	11:00 AM		5	5	5	5
	10:30 PM		5	5	5	5
12	10:30 AM		5	5	5	5
	9:30 PM		5	5	5	5
13	9:30 "		5	5	5	5
15	3:00 "		5	5	5	5
Results		Dead	Living	Living	Living	Living

before treatments were started. The 1st animal was treated immediately after being injected with the organism; the 2nd animal was given the first treatment 3 hours later; the 3rd animal was treated 6 hours later; and the 4th animal was treated 9 hours later. The subtilin preparation used was the same as in the first experiment. All treatments were given intraperitoneally. The schedule of treatments and results obtained are given in Table II.

It may be seen that the control guinea pig died in 77 hours whereas those treated with subtilin were still living after the 10th day, when the experiment was discontinued. The

guinea pig treated as late as 9 hours after being inoculated with *Bacillus anthracis* was protected from the disease. The results indicate that subtilin exerts a powerful *in vivo* action on experimental anthrax infections in guinea pigs. The antibiotic did not produce any observable toxic reaction in the animals.

Conclusions. Subtilin has been shown to produce a powerful action on the course of experimental anthrax infections in guinea pigs. Animals treated with subtilin 9 hours after being injected with the organism were protected from the infection. The antibiotic exhibited no apparent toxic reaction in the animals.