Summary. 1. As determined by the customary cup test procedure it is not possible to obtain complete recoveries of penicillin G and K when added to plasma and tissues. A considerable error is involved in the assay of penicillin K in the presence of a high concentration of plasma. Such low recoveries have been shown not to be due to destruction of penicillin K. 2. For a given dose, the plasma level of penicillin K 5 minutes after intravenous injection is less than one-half that with penicillin G. The rate of disappearance from a given plasma concentration, however, is not greatly different for the 2 penicillins. 3. The apparent volume of distribution of G is 20.2% of the body weight, whereas with K it is 57.1%. Both of the penicillins are localized in lung and liver to some extent. Penicillin K is localized to a considerably greater extent in the liver than is G. 4. As determined by dialysis experiments penicillin K is adsorbed by some component of dog plasma to a much greater extent than is penicillin G.

15656

Subtilin-Antibiotic Produced by *Bacillus subtilis*.* V. Effect on *Streptococcus pyogenes* Infections in Mice.†

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In a previous communication[1] subtilin was found to exert a pronounced antibiotic action against a large number of species of Gram-positive organisms, including *Streptococcus pyogenes* (hemolytic) by the agar cup-plate procedure. The agent was shown to be bacteriostatic in high dilution and bactericidal in more concentrated solution. Subtilin exhibited an extremely low toxicity index to living embryonic chick heart tissue fragments cultivated *in vitro*[2] and exerted a powerful *in vivo* action on the course of experimental pneumococcus infections in mice[3] and anthrax in guinea pigs.[4]

TABLE I.
Treatment of *Streptococcus pyogenes* Infections in Mice.

		6 mice used in each group				
Date	Time	Control	Treated immediately	Treated after 3 hr	Treated after 6 hr	Treated after 9 hr
8-13-46	9 a.m.	Infected	Infected	Infected	Infected	Infected
"	9 "	—	0.5 cc subtilin	—	—	—
"	12 m.	—	" " "	0.5 cc subtilin	—	—
"	3 p.m.	—	" " "	" " "	0.5 cc subtilin	—
"	6 "	—	" " "	" " "	" " "	0.5 cc subtilin
"	11 "	All dead	1.0 " "	1.0 " "	1.0 " "	1.0 " "
14	9 a.m.	—	" " "	" " "	" " "	" " "
"	12 m.	—	" " "	" " "	" " "	" " "
"	4 p.m.	—	" " "	" " "	" " "	" " "

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

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

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

4 Salle, A. J., and Jann, Gregory J., PROC. SOC. EXP. BIGL. AND MED., 1946, **63**, 41.

TABLE II.
Treatment of *Streptococcus pyogenes* Infections in Mice.

Date	Time	8 mice used in each group: Control	Treated immediately	Treated after 3 hr	Treated after 6 hr	Treated after 9 hr
9-19-46	9 a.m.	Infected	Infected	Infected	Infected	Infected
,,	9 ,,	—	0.5 cc subtilin	—	—	—
,,	12 m.	—	,, ,, ,,	0.5 cc subtilin	—	—
,,	3 p.m.	—	,, ,, ,,	,, ,, ,,	0.5 cc subtilin	—
,,	6 ,,	—	,, ,, ,,	,, ,, ,,	,, ,, ,,	0.5 cc subtilin
,,	8 ,,	1 dead	—	—	—	—
,,	9 ,,	—	1.0 cc ,,	1.0 ,, ,,	1.0 ,, ,,	1.0 ,, ,,
9-20-46	9 a.m.	All dead	0.5 ,, ,,	0.5 ,, ,,	0.5 ,, ,,	0.5 ,, ,,

In the present communication subtilin was tested for its effect on *Streptococcus pyogenes* infections in white mice.

Experimental. Thirty white mice, weighing between 20 and 25 g were injected intraperitoneally with 0.2 cc of a saline suspension of a 24-hour brain heart infusion agar slant culture of *Streptococcus pyogenes*. The turbidity of the saline suspension was adjusted to correspond to a No. 3 MacFarland nephelometer standard.

The animals were divided into 5 groups and treated as follows: mice in Group 1 were not treated but served as controls; mice in Group 2 were treated immediately after infection; mice in Group 3 were treated 3 hours after infection; mice in Group 4 were treated 6 hours after infection; and the mice in the last group were treated 9 hours after infection. Each mouse was injected intraperitoneally with 0.5 cc of a solution containing 0.2 mg subtilin per cc (1 unit)[2] and every 3 hours thereafter. This dose was increased to 1 cc (2 units) at the end of the first day and throughout the next.

The schedule of treatments and results obtained are given in Table I. It may be seen that all of the control animals died within 14 hours whereas all of the treated animals survived and were apparently normal 2 weeks after treatment was discontinued. The mice were discarded after this period of time.

The animals treated 9 hours after being infected were 5 hours from death and appeared to be beyond recovery. The infection was accompanied by diarrhea, ruffled fur, and prostration. After receiving 2 injections of subtilin, the diarrheal condition disappeared and the animals appeared to be almost normal. After the fourth injection, the animals looked and acted like healthy mice and devoured food freely. The recovery was so spectacular that it was almost beyond belief.

In a second series a larger number of mice (40) was used to check the results in the first experiment. The method was the same but the amount of subtilin administered was less. The results are recorded in Table II.

It may be seen that one of the untreated mice died within 11 hours. The others died within 24 hours but the exact time could not be ascertained because death occurred sometime during the first night. On the other hand, all of the animals given subtilin survived and were healthy and normal 2 weeks after treatment was discontinued. The animals were sacrificed after this period of time. Examination of the hearts' blood of animals dead of the disease showed the presence of large numbers of *Streptococcus pyogenes*. The antibiotic did not produce any observable toxic reaction in the mice.

Conclusions. Subtilin has been shown to exert a powerful *in vivo* action on the course of experimental *Streptococcus pyogenes* infections in mice. Animals given subtilin 9 hours after being infected were in such an advanced stage of the disease at the time treatment was started that recovery was almost unbelievable. The antibiotic exhibited no apparent toxic reaction in the mice.