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Observations on the Mechanism of Action of Penicillin.*

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In previous reports[1, 2] the preparation and *in vitro* activity of penicillin was described. Studies on its mode of action are herewith reported.

Rate of Killing. Pneumococci, hemolytic streptococci and staphylococci were tested with penicillin under various conditions, the number of surviving organisms per cc being determined at intervals. It was found that the number of survivors decreased by geometric units as time increased by arithmetic units. The log of the number of survivors plotted against time followed a straight line until at least 99% of the organisms were killed. Under the experimental conditions, pneumococci were destroyed at a more rapid rate than hemolytic streptococci, and hemolytic streptococci more rapidly than staphylococci. (Graph I.)

Furthermore, with a given concentration of penicillin the rate of killing of a given strain of hemolytic streptococci decreased as the number of organisms present at 0 hours increased. Likewise, with a given number of hemolytic streptococci the rate of killing increased within limits as the concentration of penicillin was increased. There was a point, however, beyond which, with a constant number of hemolytic streptococci per cc, increases in the concentration of penicillin no longer increased the rate of killing. (Graph II.)

The killing of hemolytic streptococci at a constant rate applied only to the destruction of approximately 99% of the organisms originally present. With the last 1% of the organisms, the reaction followed one of 3 courses: (1) The number of organisms continued to decrease at the same rate until complete sterilization had been effected; (2) the number decreased at a somewhat slower rate, or, (3) the number increased. It is therefore apparent that penicillin

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1 Hobby, G. L., Meyer, K., Chaffee, E., PROC. SOC. EXP. BIOL. AND MED., 1942, **50**, 277.

2 Meyer, K., *et al.*, *Science*, 1942, in press.

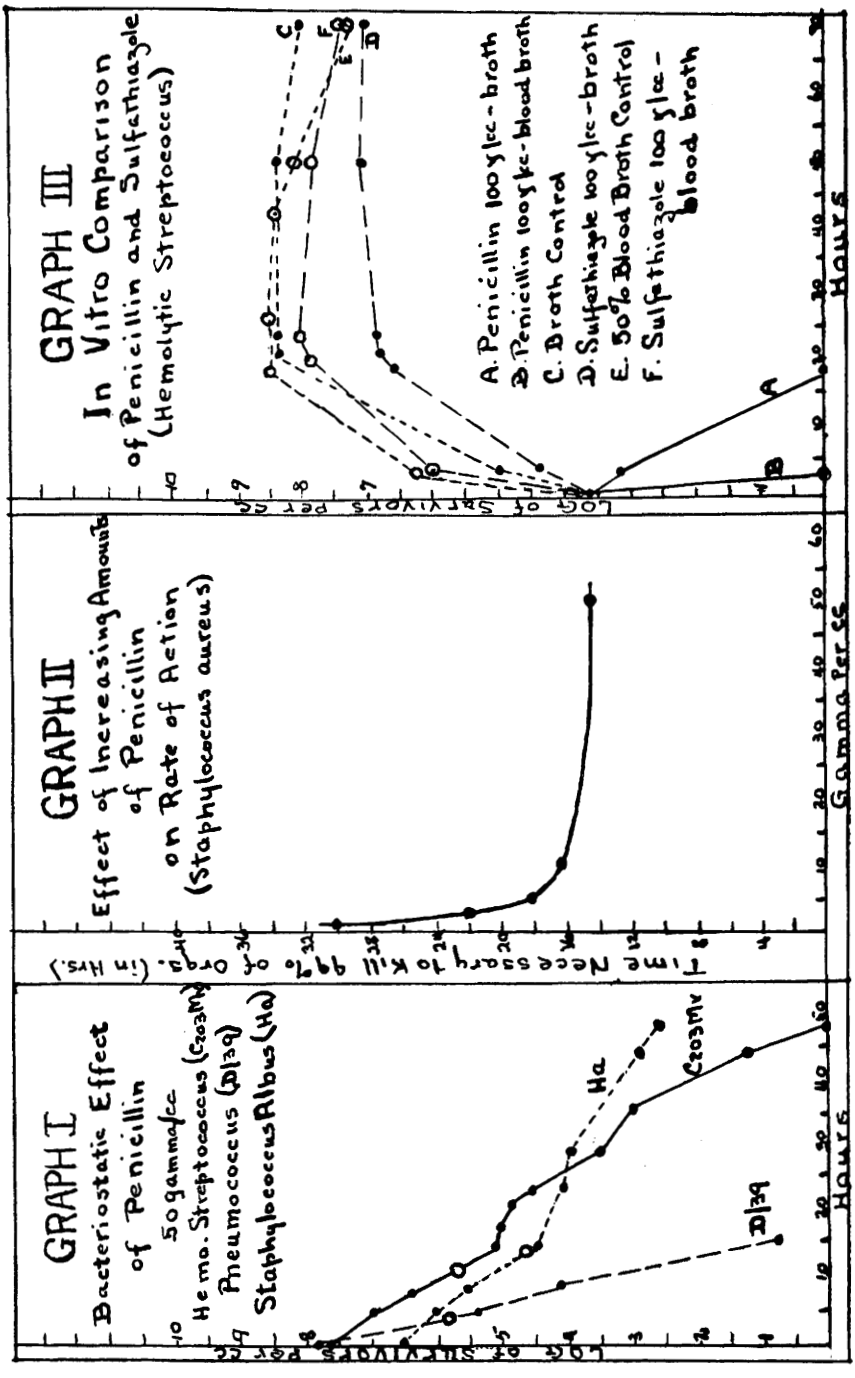
GRAPH I
Bacteriostatic Effect of Penicillin
50 gammas/cc
Hemo. Streptococcus (C203M)
Pneumococcus (D139)
Staphylococcus Albus (Ha)
Log of Survivors per cc
Hours
D139
Ha
C203M
GRAPH II
Effect of Increasing Amounts of Penicillin on Rate of Action (Staphylococcus aureus)
Time necessary to Kill (in Hrs.)
Gamma Per cc
GRAPH III
In Vitro Comparison of Penicillin and Sulfathiazole (Hemolytic Streptococcus)
Log of Survivors per cc
Hours
A. Penicillin 100 γ/cc - broth
B. Penicillin 100 γ/cc - blood broth
C. Broth Control
D. Sulfathiazole 100 γ/cc - broth
E. 50% Blood Broth Control
F. Sulfathiazole 100 γ/cc - blood broth

TABLE I.
Comparison of Penicillin, Gramicidin, and Tyrocidin *in Vitro*.

Culture	Inhibiting agent*	No. of viable organisms per cc × 1000				
		0 hr	1 hr	3 hrs	7 hrs	24 hr
Streptococcus hemolyticus (C203 Mv)	Penicillin	1,500	4,300	2,650	420	0
	Gramicidin	1,500	2,430	1,140	7	2.4
	Tyrocidin	1,500	0.1	0	0	0

*10 γ per cc of each was used.

may act as a bacteriostatic agent or it may induce a true bactericidal effect.

The action of penicillin is in sharp contrast to that of the sulfonamides. Whereas penicillin produces an actual killing effect, the sulfonamides cause only a slowing up of the rate of growth. (Graph III.)

The activity of penicillin is comparable to that of gramicidin and tyrocidin. Penicillin, gramicidin, and tyrocidin all prevent multiplication and bring about a definite decrease in the number of organisms per cc. However, penicillin acts more slowly than either gramicidin or tyrocidin. (Table I.)

Lysis. To heavy suspensions of hemolytic streptococci, penicillin was added in concentrations as high as 100 γ per cc. No lysis of the organisms was observed on incubation at 37°C for as long as 24 hours.

Absorption. Broth containing penicillin was seeded with hemolytic streptococci (C203 Mv) in such a way as to give a final culture dilution of 10^{-2}. The cultures were incubated at 37°C and the number of organisms per cc counted at 0 and at 48 hours. At 0 and at 48 hours, portions were also filtered through a Seitz filter and the filtrates tested for penicillin activity. A definite decrease in the number of organisms per cc occurred, but no diminution in the amount of penicillin was detected. (Table II.)

In further experiments, 350 cc amounts of culture were centrifuged and the organisms suspended in 2 cc of broth. Four cc of

TABLE II.
Absorption of Penicillin by Bacteria. No. Organisms × 1000.

No. of hours	Control Orgs. per cc	Penicillin	
		Orgs. per cc	Titer of supernatant
0	3,000	3,000	0.1 γ/cc
48	0	0	0.1 "
0	2,950,000	2,950,000	0.8 "
24	410	74	1.0 "

broth containing penicillin was added to 1 cc of this suspension, giving a final penicillin concentration of 340 γ per cc. Four cc of broth was added as control to 1 cc of the suspension of organisms. As in the previous experiment, the cultures were incubated at 37°C and the number of organisms per cc determined at intervals. At the same intervals portions were filtered and the filtrates tested for penicillin activity. Under these conditions the number of viable organisms remained unaltered and no loss of penicillin activity was observed. (Table II.)

Effect of Growth Phase on Activity of Penicillin. Undiluted 18-hour broth cultures of hemolytic streptococci (C203 Mv) were divided into 6 equal portions. To each of 3 samples, 0.1 cc of a solution of penicillin was added giving a final concentration of 100 γ per cc. To each of the remaining 3, 0.1 cc of broth was added. One tube from each set was incubated at 4°C, at 18°C, and at 37°C. The number of organisms per cc was determined at 0 and at 48 hours.

No multiplication took place in the control broth tubes either at 4°, 18°, or 37°C. At the same time no decrease in the number of organisms per cc occurred in the cultures to which penicillin was added although its activity remained unchanged. (Table III.)

Similar experiments were carried out using cultures containing fewer organisms per cc. These results are also illustrated in Table III. At 4°C no multiplication occurred in the control broth. At this temperature penicillin showed no bacteriostatic or bactericidal action on the organisms. At 18°C multiplication occurred at a slow rate and penicillin showed moderate antibacterial activity. At 37°C multiplication was rapid and the antibacterial action of penicillin was marked. It is apparent that penicillin is capable of destroying bacteria only if multiplication takes place. In similar experiments carbolic acid in a concentration of 0.1% was also effective only under conditions suitable for multiplication.

Summary. Penicillin acts either as a bacteriostatic or bactericidal

TABLE III.
Effect of Temperature and Concentration of Organisms on Penicillin Activity.
No. of Organisms × 1000.

Medium	Organisms per cc at 0 hr	Organisms per cc at 48 hours		
		4°C	18°C	37°C
Broth penicillin, 100 γ/cc	5,500	6,480	17,000	130,000
	5,500	5,280	320	0.1
Broth penicillin, 100 γ/cc	94,000	90,000	67,500	85,000
	94,000	92,000	60,000	126,000

agent depending on the experimental conditions. The number of organisms decreases at a constant rate until 99% of the organisms have been destroyed. The rate of killing varies with different organisms. The action of penicillin on hemolytic streptococci is not accompanied by lysis of the organisms. No detectable amount of penicillin is destroyed or absorbed from solution by the organisms. It appears to be effective only when active multiplication takes place.

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Chemotherapeutic Activity of Penicillin.*

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The activity of penicillin *in vitro* has been discussed in detail in previous communications.[1] Its *in vivo* activity was first reported by Chain, *et al.*,[2] and by Florey, *et al.*[3]

In vivo Activity. Mice infected intraperitoneally with varying amounts of a highly virulent strain of hemolytic streptococci (C203 Mv) were treated subcutaneously with small amounts of penicillin.

TABLE I.
Effect of Subcutaneous Injection of Penicillin on Group A Hemolytic Streptococcus Infections (C203 Mv).

Dilu. of culture	No. of orgs. inj. ×1000	No. of mice	Amt. of penicillin,* cc	No. of days treated	No. dead (<48 hrs)	No. prolonged life (2-7 days)	No. survived (>7 days)
2.0cc	180,000	9	.4-.65	<6	3	1	5
1.0cc	90,000	10	.4-.60	<6	1	2	7
10^{-1}	9,000	15	.2-.58	<6	2	4	9
10^{-2}	900	13	.2-.57	<6	0	4	9
10^{-3}	90	11	.2-.57	<6	0	3	8
Controls							
10^{-5}	0.9	15	0	0	15	0	0

*Alcoholic solution: Total dosage per mouse calculated to be <7 mg dry weight.

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We are indebted to Dr. Martin H. Dawson for his valuable help and suggestions throughout this work.

1 Hobby, G. L., Meyer, K., Chaffee, E., PROC. SOC. EXP. BIOL. AND MED., 1942, **50**, 277, 281.

2 Chain, E., *et al.*, *Lancet*, 1940, **2**, 226.

3 Florey, H. W., *et al.*, *Lancet*, 1941, **2**, 177.