all resistant strains, both Gram-positive and Gram-negative, and not in sensitive ones.

Summary. The effect of penicillin *in vitro* varies with the concentration of penicillin, the number of organisms present, and the rate of growth of the organisms. It is most effective when rapid multiplication takes place.

Penicillin is inhibited by a substance found in resistant strains of bacteria and not in the sensitive strains tested. The presence of this inhibiting substance in resistant strains and its absence in sensitive strains suggests that resistance to penicillin may depend upon the capacity of an organism to elaborate this substance. The degree of sensitivity of any strain not producing the inhibiting substance appears to be correlated with the rate of growth exhibited by that strain.

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Effect of Rate of Growth of Bacteria on Action of Penicillin.*

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In previous communications[1, 2] the effect of growth conditions of hemolytic streptococci on the action of penicillin were described. In the present report further observations of this nature will be reported.

Experimental. Hemolytic streptococcus (strain C203MV) was used throughout. Unless otherwise specified organisms were grown in a beef infusion phosphate buffered medium.

1. Conditions which enhance growth: Hemolytic streptococci were incubated in plain broth, in broth containing varying concentrations of defibrinated rabbits' blood, and in whole defibrinated rabbits' blood. A constant amount of penicillin was added to each of these. The number of organisms per cc was determined at various intervals.

In the presence of defibrinated rabbits' blood there was an increase in the rate of growth of hemolytic streptococci and a corresponding increase in the rate at which the organisms were destroyed by penicillin. The effect of 100% defibrinated rabbits' blood in contrast to plain broth is shown in Table I.

In a further series of experiments, the effect of serum, para-aminobenzoic acid, and dextrose was observed. Each of these substances also increased the rate of growth of hemolytic streptococci and caused a corresponding increase in the rate at which the organisms were destroyed by penicillin.

2. Conditions which inhibit growth: Hemolytic streptococci were incubated in media containing a constant amount of penicillin and varying concentrations of physiological saline. One volume of nutrient broth was diluted with one and with two volumes of physiological saline. A constant amount of penicillin and a constant number of organisms per cc were added. The number of organisms per cc was again determined at various intervals. Under these conditions there was a decrease in the rate of growth of hemolytic streptococci. There was likewise a decrease in the rate at which the organisms were destroyed by the penicillin. (Table II.)

Similar experiments were carried out in broth containing both penicillin and sulfadiazine. Sufadiazine was added to nutrient broth in amounts sufficient to give a concentration of 100 μg per cc. Penicillin was then added giving a concentration of 6.4 Oxford units per cc. Controls were run with nutrient

* This work was presented in part before the New York and New Jersey Branches of the Society of American Bacteriologists, December, 1942.

1 Hobby, G. L., and Dawson, M. H., PROC. SOC. EXP. BIOL. AND MED., 1944, **56**, 178.

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

TABLE I.
Effect of Blood on Rate of Action of Penicillin.

Medium	Conc. of penicillin (Oxford units/cc)	No. of organisms (× 1000) per cc			
		0 hr	2 hr	4½ hr	7 hr
Blood*	—	8200	69000	900000	880000
"	3.34	8200	200	165	84†
Plain broth	—	8200	19000	84000	60000
" "	3.34	8200	8000	1180	79†

* 100% defibrinated rabbit's blood.
† 0 per cc at 30 hours.

TABLE II.
Effect of Sodium Chloride on Rate of Action of Penicillin.

Amount of saline in broth %	Concentration of penicillin (Oxford units/cc)	No. of organisms (× 1000) per cc				
		0 hr	2¼ hr	4½ hr	7 hr	24 hr
—	—	6345	32000	152000	190000	215000
—	—	6345	1950	1400	12	0
50	—	6345	30500	127000	135000	185000
50	5	6345	3900	1740	230	0
66	—	6345	8800	54500	75300	245000
66	5	6345	3750	2700	690	200

broth alone, nutrient broth plus sulfadiazine, and nutrient broth plus penicillin alone. A 10^{-3} dilution of a 15 hour broth culture of hemolytic streptococci (strain C203MV) was then added, giving a final dilution of 10^{-4}. The number of organisms per cc was again determined at intervals.

During the first 5 to 7 hours of incubation, sulfadiazine alone had no effect on the rate of growth of hemolytic streptococci. During the same period penicillin alone destroyed about 90% of the organisms originally present. When incubation was continued further penicillin gradually destroyed the remaining viable organisms. During the first 5 to 7 hours, the combination of penicillin and sulfadiazine together killed at the same rate as penicillin alone. After 5 to 7 hours, the combination of penicillin and sulfadiazine destroyed the remaining organisms more slowly than did penicillin alone.

In another series of experiments hemolytic streptococci were incubated in the presence of sulfadiazine for 5 to 7 hours and then penicillin was added. Controls were prepared with nutrient broth alone, with nutrient broth containing sulfadiazine, and with nutrient broth to which penicillin was added after 5 to 7 hours' incubation.

The results again indicated that the presence of sulfadiazine caused a decrease in the rate of growth after 5 to 7 hours. Penicillin added to the sulfadiazine-containing medium during this phase of slow multiplication destroyed the organisms more slowly than when added to plain broth at the same stage in the growth cycle.

Further experiments were carried out under conditions which allowed little or no multiplication of the organisms. The effect of penicillin on varying concentrations of hemolytic streptococci, incubated at 4° C was determined. Varying dilutions of an 18-hour culture were employed. The undiluted culture contained 200,000,000 organisms per cc, and the dilutions 2,000,000 and 40 organisms per cc respectively. Penicillin was added to each dilution in amounts varying from 0.0009 to 0.9 Oxford units per cc and the cultures were incubated at 4° C. Controls without penicillin were incubated simultaneously. The number of organisms per cc at various intervals was determined by colony counts.

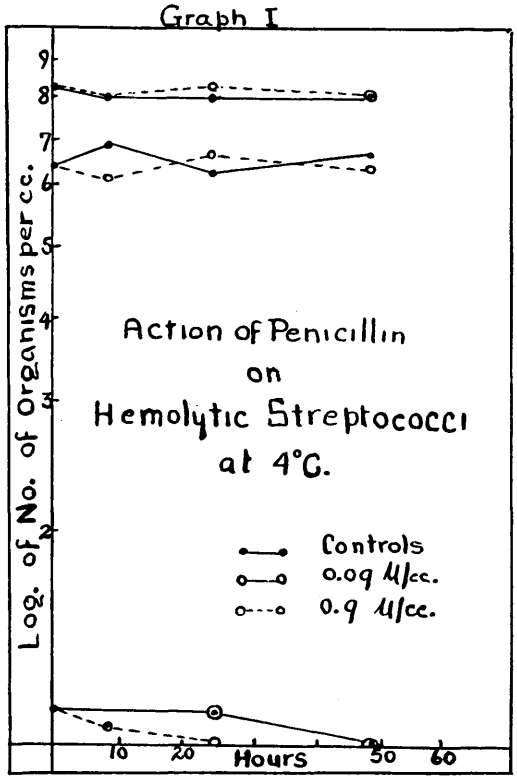
At 4° C no multiplication occurred regardless of the initial concentration of organisms. With an initial concentration of 200,000,000

TABLE III.
Effect of Medium on the Action of Penicillin.

Temp. of incub.	Time in hrs	No. of organisms (× 1000) per cc: Oxford units of penicillin per cc 0	0.1	10.0	100.0
		Unwashed Cells.			
37°C	0	92000	92000	92000	92000
	24	240000	145000	78000	64000
	48	19950	2750	805	1805
4°C	0	92000	92000	92000	92000
	24	190000	160000	245000	230000
	48	164000	103000	136000	130000
		Washed Cells.			
37°C	0	93000	93000	93000	93000
	24	505000	505000	1350	1350
	48	163000	69000	920	540
4°C	0	93000	93000	93000	93000
	24	170000	150000	175000	215000
	48	232000	121000	138000	140000

or 2,000,000 organisms per cc no killing occurred with amounts of penicillin up to 0.9 Oxford units per cc. With fewer organisms a gradual decrease in the number of organisms occurred with 0.9 units of penicillin per cc but not with 0.09 units per cc. (Graph I).

Graph I



In further experiments an 18-hour culture of hemolytic streptococci containing 93,000,000 organisms per cc was divided into two portions. One portion was centrifuged, the organisms washed 3 times with sterile broth and resuspended in an equal volume of broth. The other portion remained unwashed. Each was then subdivided into 4 portions. One of the 4 was kept as a control and varying concentrations of penicillin were added to each of the remaining 3. Incubation was carried out at 37° C and at 4° C.

The unwashed and washed cultures showed little multiplication in plain broth at 4° C; likewise they were unaffected by large amounts of penicillin at this temperature.

At 37° C little multiplication occurred in the unwashed cultures in plain broth; only a slight decrease in the number of organisms took place in the presence of relatively large amounts of penicillin. Washed organisms suspended in fresh broth also multiplied only to a slight extent. Under the influence of large amounts of penicillin the organisms were destroyed. However the destruction in washed suspensions proceeded more rapidly than that in the unwashed cultures. (Table III). It would seem that unwashed cultures contain some factor which inhibits the action of penicillin.

Observations showed that penicillin did not cause lysis of bacteria under conditions per-

mitting minimal multiplication. No absorption of penicillin onto the organisms could be demonstrated. Incubation of small numbers of organisms with penicillin under conditions suitable for active growth, however, resulted in the disappearance of the organisms. The failure to demonstrate these killed organisms by the usual staining technics suggested that they had undergone a subsequent lysis. This is in accord with the observations of other workers.[3, 4]

Summary. Conditions which increase the rate of growth of bacteria increase the rate at which penicillin acts. Conditions which decrease the rate of growth decrease also the rate at which penicillin acts. Penicillin is most effective when active multiplication takes place.

3 Fleming, A., *Brit. J. Exp. Path.*, 1929, **10**, 226.
4 Chain, E., *et al.*, *Lancet*, 1940, **2**, 226.

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Relationship of Penicillin to Sulfonamide Action.

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Ungar[1] reported on the synergistic action of both para-aminobenzoic acid and sulfapyridine on penicillin. In a previous communication from this laboratory,[2] it was stated that para-aminobenzoic acid increased the rate of action of penicillin whereas sulfadiazine decreased the rate of its action.

Experimental. Unless otherwise specified the Oxford strain of *Staphylococcus aureus* (strain H) grown in Knight's synthetic medium was used throughout the present study. Before use the culture was transferred 3 times in Knight's basic medium to which had been added M/100 nicotinic acid. A solution of penicillin was diluted serially in Knight s medium containing 100 μg of para-aminobenzoic acid per cc, in Knight's medium containing 20 μg of sulfadiazine per cc, and in Knight's medium containing 20 μg of sulfapyridine per cc, respectively. *Staphylococcus aureus* was inoculated into each tube in an amount sufficient to give a final dilution of 10^{-2} or approximately 10,000,000 organisms per cc. Incubation was carried out at 37°C for 24 hours. The last tube in which no visible growth occurred was accepted as the titer of the penicillin solution.

The titer of the penicillin in Knight's medium was 0.035 Oxford units per cc. With sulfapyridine the titer increased twofold to 0.018 units per cc. With para-aminobenzoic acid or sulfadiazine no increase was observed. (Table I.)

A similar experiment was carried out using hemolytic streptococci (strain C203MV) grown in beef infusion broth. No increase in the titer of the penicillin was observed with sulfadiazine, sulfapyridine, or para-aminobenzoic acid. (Table I.)

It was apparent that, although para-aminobenzoic acid and the sulfonamides may affect the rate of action of penicillin, they do not significantly affect the titer of penicillin.

Further preliminary experiments were carried out to determine any possible relationship between the action of penicillin and that of sulfadiazine or sulfapyridine.

West and Coburn[3] showed that sulfapyridine is capable of exerting a bacteriostatic

1 Ungar, J., *Nature*, 1943, **152**, 245.
2 Hobby, G. L., and Dawson, M. H., PROC. SOC. EXP. BIOL. AND MED., 1944, **56**, 178.
3 West, R., and Coburn, A. F., *J. Exp. Med.*, 1940, **72**, 91.