in the Syrian hamster *in situ*. The organisms penetrate along selected channels and appear to invade and utilize the organic matrix in satisfying their metabolic requirement.

Molar II was inoculated with strain #82, isolated from scrapings of a microscopic human carious lesion. This strain was also highly pleomorphic. The pH of the culture, when tested, was in the neighborhood of 7.1. On the 75th day of incubation the molar was removed from the culture and placed in the fixative. An examination of the culture at this time showed it to be contaminated. It is presumed that the contamination occurred when the broth was changed 5 days earlier, since it was uncontaminated at that time. In the histological preparation of this tooth a considerable amount of the matrix was lost. The fragments which were recovered, however, exhibited a penetration by filamentous forms similar to that seen in Molar I.

It is admitted that the *in vitro* experiments to date are too few to make a definite conclusion, but we feel that the studies advance a method by which further studies may be conducted. The observations indicate that there may be an etiological relationship between the oral actinomycetes and caries of the enamel. The *in vitro* work is being repeated, using the same strains of organisms. Other strains will also be employed and other oral bacteria will be tested by the above method.

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A Paradoxical Zone Phenomenon in the Bactericidal Action of Penicillin *in Vitro*

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If varying concentrations of penicillin are added to suspensions of bacteria *in vitro*, and if at intervals thereafter one determines the number of organisms surviving at each concentration, one usually finds that the susceptibility of the particular organisms can be defined in terms of three concentrations of penicillin: (1) the concentration which serves only to reduce the rate of multiplication, (2) a somewhat higher concentration at which the organisms die faster than they multiply, with a slow decrease in the number of viable organisms, and (3) a maximally effective concentration, which varies between 2 and 10 times the "sensitivity level" of the organism as ordinarily defined. At this optimal concentration of penicillin the organisms are killed at a maximal rate which is not further affected even by a 20,000-fold increase in the concentration of the drug.

These relationships are illustrated in Table 1 for the C-203 strain of *Streptococcus pyogenes*. Different species of bacteria, and different strains of the same species, vary with respect to both the effective concentrations of penicillin and the maximum rate at which the organisms can be killed. Qualitatively, however, with most strains so far studied, there is a maximally effective level as defined above.

Paradoxical and as yet unexplained results have been obtained with certain strains of streptococci and staphylococci. With these strains there were the same three critical concentrations of penicillin, including a concentration at which the organisms were killed at a maximal rate. With further increase in the concentration of penicillin, however, the rate of bactericidal action did not remain constant as it did with, for example, *Treponema pallidum*, *Diplococcus pneumoniae*, and the group A strains of *Streptococcus pyogenes* so far tested, but instead was strikingly reduced. This is illustrated by the experiment of Table 2, carried out with the Smith strain of *Staphylococcus aureus*. In that experiment, after, for example, 6 hrs exposure to penicillin at varying concentrations, there were 400 times as many viable organisms at a concentration of 2,048 μg/cc than there were at the optimum concentration of 0.096 μg/cc, and it required 27 hrs instead of 5 to kill 99.9% of the organisms.

To test the regularity of this phenomenon, 13 strains of β-hemolytic streptococci (Lancefield groups A, B, C), 7 strains of *Str. faecalis*, 4 other strains of α-hemolytic streptococci, 7 strains of *Staph. aureus*, and 2 strains of *Staph. albus* were tested by the same technic.

Five of the 7 strains of *Str. faecalis* were rapidly killed at an optimal concentration of 4 μg/cc, at which 99.9% of the organisms were rendered nonviable in 5–7 hrs. All 5 of these strains were killed much more slowly at higher concentrations of penicillin. Thus, at 512 μg/cc it required 36–46 hrs to kill 99.9% of the organisms, instead of 5–7 hrs. This zone phenomenon was equally apparent if the results were expressed in terms of the proportion surviving after, for example, 6 hrs. Three of the other 4 strains of α-hemolytic streptococci tested showed a similar zone.

Of the β-hemolytic streptococci, the 5 group A strains failed to show the zone phenomenon. However, all 4 of the group B strains, and 2 of the 4 group C strains tested, showed a zonal susceptibility to penicillin resembling that illustrated in Table 2. Three of the 7 strains of *Staph. aureus* tested, and 1 of the 2 strains of *Staph. albus* showed the zone phenomenon in varying degree.

The paradoxically reduced bactericidal activity of penicillin in high concentrations was not due to the appearance of resistant strains at the higher concentrations. With *Str. faecalis*, the last few organisms surviving from an initial inoculum of approximately 1,000,000/cc, when subcultured and tested by the same technic as the original strain, proved to have the same resistance as the original culture, judged either by the effective concentrations of

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penicillin or by the rate of death at the optimal concentration; and with such cultures there was the same zone of diminished activity at high concentrations of penicillin. The similar survivors in the case of the Smith strain of *Staph. aureus* were significantly more resistant than the original strain, but did not differ in this respect from rate at which the organisms were killed at high and low concentrations of penicillin.

The phenomenon here described may be of therapeutic significance if it should prove that, *in vivo* also, high concentrations of penicillin are less actively bactericidal against certain strains of staphylococci and streptococci

TABLE 1

EFFECT OF THE CONCENTRATION OF PENICILLIN G ON THE RATE AT WHICH *Str. pyogenes* (C-203) IS KILLED *in Vitro**

Time at 37°C (hrs)	Concentration of penicillin (μg/cc)									
	0	0.004	0.006	0.008	0.012	0.016	0.024	0.032	0.064	512
	% of organisms surviving (referred to original inoculum as 100)									
1½	.....	.....	....	.....	10.0	0.88	0.28	0.12	0.063	0.04
3	3,300	1,400	680	100	1.0	0.03	0.02	0.013	0.008	0.007
6	15,000	1,700	21.6	4.7	0.02	0.006	0.0038	0.003	0.002	....
12	.....	.....	....	0.024	0.0012	0.0003	0.00015	0.00015	0	....
24	4,500	15.3	0.5	0.002	0	0	0	0	....	....
Time required to kill 99% of organisms (hrs)		> 24	> 24	11	4.8	2.5	2.2	1.9	1.4 ±	1.4 ±

* Original inoculum = 750,000/cc (by colony count).

organisms which had survived the low optimal concentration of 0.064–0.128 μg/cc.

The diminished bactericidal activity in the presence of excess penicillin was not due to the production of free penicillinase. The supernatant fluid from bacterial suspensions exposed to high concentrations of penicillin for 12 to 24 hrs contained as much penicillin as an uninoculated control tube, similarly incubated. Further, if strains of *Staph. aureus* or of *Str. faecalis* were cultured in the presence of amounts of penicillin so small as not to prevent multiplication, that minimal concentration of penicillin was found to have been unaffected even after cultivation for 24 hrs, again as compared with an uninoculated control tube under similar conditions of incubation.

The zone phenomenon was found to be related to the number of organisms inoculated. The larger the inoculum, the more pronounced was the difference in the

TABLE 2

EFFECT OF THE CONCENTRATION OF PENICILLIN G ON THE RATE AT WHICH A STRAIN OF *Staph. aureus* IS KILLED *in Vitro**

Time at 37°C (hrs)	Concentration of penicillin (μg/cc)										
	0	0.024	0.032	0.048	0.064	0.096	0.128	0.25	1.0	4.0	2,048
	% of organisms surviving (referred to original inoculum as 100)										
3	12,500	200	240	27	6.4	0.8	0.56	1.1	16.4	16.6	12.0
6	11,500	230	42	0.06	0.045	0.03	0.036	0.34	5.2	9.0	12.5
12	19,800	....	0.19	0.0036	0.0038	0.0032	0.0054	0.043	0.8	1.25	1.5
24	37,100	340	280	0.031	0.075	.....	0.0005	0.0014	0.021	0.022	0.15
48	40,300	1,530	865	.....	.....	.....	0.00013	0.00026	0.00026	0.00013	0.0011
Time required to kill 99.9% of organisms (hrs)		....	13 ±	5.8	5.5	5	5	9.5	19	20	27

* Original inoculum = 2.7×10^6/cc (by colony count).

than are lower concentrations of the drug. When penicillin in aqueous solution is injected intramuscularly, the optimal concentration would be present in the tissues for a relatively short period of time. Immediately after the injection, the penicillin might be present in concentrations far exceeding this optimal concentration, and correspondingly less active; because of the speed with which penicillin disappears from the blood and tissues, the optimal concentration would be present for a relatively brief period; and as soon as the penicillin level in the tissues falls below that optimal concentration, the rate of bactericidal action would be correspondingly reduced. With such strains, the most effective method for the administration of penicillin might well be a continuous intravenous or intramuscular infusion at a rate designed to maintain, at the foci of infection, concentrations of penicillin approximating the maximally effective level for the particular organism.