

and West,¹⁰ could have been removed by the centrifugation.

Summary. 1. By special handling and prolonged centrifugation of normal blood, plasmas with a delayed clotting time can be obtained. If centrifugation is prolonged sufficiently, the

plasmas become spontaneously incoagulable.

2. Normal plasmas of this type require the presence of platelets and perhaps other formed elements to correct the delayed clotting of hemophilic plasma.

3. These findings indicate that in hemophilia there is a deficiency in a plasma factor required for platelet utilization. It is suggested that this factor is a thrombocytolysin.

¹⁰ Chargaff, E., and West, R., *J. Biol. Chem.*, 1946, **166**, 189.

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Effect of Penicillin on the Tubercle Bacillus *In vitro*.

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Reports concerning the effect of penicillin on the tubercle bacillus are contradictory. The Oxford group originally noted no inhibition of growth in glycerol broth with a penicillin concentration of 40 units per cc.¹ Subsequently, inhibition by as little as 20 units per cc was observed by one investigator,² whereas others reported no inhibition with concentrations of 20 to 30 units per cc.^{3,4} Actual stimulation of growth by small concentrations of penicillin was described in one instance,⁵ while in another, rapid destruction of large amounts of penicillin (800 units per cc) by culture filtrates of *M. tuberculosis* was observed.⁶ These contradictory results, and the desirability of using penicillin to suppress the growth of contaminants in cultures for the primary isolation of tubercle bacilli, have made it appropriate to reinvestigate the effect of penicillin on the tubercle bacillus *in vitro*.

Materials. H37Rv, a standard virulent hu-

man strain of *M. tuberculosis*, was used throughout the experiments because of its similarity in virulence and response to antimicrobial agents to strains isolated from patients with tuberculosis.^{7,8}

The experiments were performed in liquid and on solid media recently developed in this laboratory.^{9,10} The penicillin employed was commercial crystalline penicillin G, obtained from various manufacturers.

Experimental. Lytic Action of Penicillin. A large inoculum of tubercle bacilli, 0.25 mg per cc, was added to test tubes 25 by 150 mm each containing 15 cc of Tween-albumin medium and penicillin in concentrations ranging from 100 to 1000 units per cc. The tubes were incubated at 37°C, and optical densities were recorded on the Coleman Spectrophotometer every day or two. Because of the possible destructive action of the tubercle bacillus on penicillin, the original concentrations were added to one set of tubes every 3 days on 4 occasions. To another set, no further penicillin was added to the amounts pres-

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¹ Abraham, E. P., *et al.*, *Lancet*, 1941, **2**, 177.

² Iland, C. N., *J. Path. and Bact.*, 1946, **58**, 495.

³ Smith, M. I., and Emmart, E. W., *Pub. Health Rep.*, 1944, **59**, 417.

⁴ Friedmann, I., *Tubercle*, 1945, **26**, 75.

⁵ Ungar, J., and Muggleton, P., *J. Path. and Bact.*, 1946, **58**, 501.

⁶ Woodruff, H. B., and Foster, J. W., *J. Bact.*, 1945, **49**, 7.

⁷ Middlebrook, G., and Yegian, D., *Am. Rev. Tuberc.*, 1946, **54**, 553.

⁸ Feldman, W. H., and Hinshaw, H. C., *Am. Rev. Tuberc.*, 1947, **55**, 428.

⁹ Dubos, R. J., and Davis, B. D., *J. Exp. Med.*, 1946, **83**, 409.

¹⁰ Dubos, R. J., and Middlebrook, G., *Am. Rev. Tuberc.*, in press.

ent at the beginning of the experiment.

Results of a representative experiment are presented in Table I. Concentrations up to 200 units per cc produced little or no inhibition of growth when no further penicillin was added. When penicillin was added at 3 day intervals, inhibition was noted in the tube originally containing 200 units per cc, indicating that penicillin inactivation was relatively slight, and that the final concentration was considerably in excess of 200 units per cc. With concentrations above 600 units per cc there was not only inhibition, but an actual decrease in optical density, indicative of lysis of the organisms.

A secondary outgrowth of the inhibited and partly lysed organisms was observed in the tubes containing 600 and 800 units per cc, when no more penicillin was added, but no secondary outgrowth occurred with a concentration of 1000 units per cc.

Relation of Inoculum Size and Medium to Penicillin Inhibition. Tubes containing 5 cc of liquid Tween-albumin or oleic acid-albumin media with various concentrations of penicillin were inoculated with tenfold dilutions of a 7-day-old culture of H37Rv diluted in 0.5 cc of distilled water. The inoculum for one set of tubes had been grown in Tween-albumin medium (dispersed growth), and for another set in oleic acid-albumin medium (granular growth). To allow for possible penicillin inactivation, 2 sets of tubes were inoculated. To one set, penicillin in the original concentrations was added on 3 occasions at 3-day intervals; to another no further penicillin was added to the amounts present at the beginning of the experiment. The results were recorded after 14 days incubation, complete inhibition of growth being regarded as the endpoint.

As shown in Table II, the inhibitory effect of penicillin was much greater in the Tween-albumin medium than in the oleic acid-albumin medium, and this difference was related to the size of the inoculum. In the oleic acid-albumin medium, growth was inhibited by 1000 units per cc; with 100 units per cc, no

TABLE I.
Bacteriostasis and Lysis of a Large Inoculum of Tubercle Bacilli by Penicillin in Tween-Albumin Medium.

A. Original Amounts of Penicillin Added 4 Times at 3-day Intervals.

Days	1	4	8	13	17
Penicillin concentrations, units per cc					
0	.082*	.156	.252	.366	.41
100	.082	.155	.222	.301	.40
200	.082	.125	.201	.275	.318
400	.082	.082	.061	.061	.061
600	.082	.061	.051	.051	.051
800	.082	.061	.051	.051	.051
1000	.082	.055	.051	.051	.051

B. No Penicillin Added to Initial Concentrations.

Days	1	4	8	13	17
Penicillin concentrations, units per cc					
0	.082*	.156	.252	.376	.43
100	.082	.156	.252	.376	.43
200	.082	.136	.252	.356	.41
400	.082	.102	.214	.346	.41
600	.082	.082	.175	.327	.41
800	.082	.061	.055	.097	.20
1000	.082	.055	.055	.055	.055

* Turbidities (optical densities) were measured with a Coleman spectrophotometer.

inhibition occurred, growth being as good as in the control tubes, regardless of the size of the inoculum. With the Tween-albumin medium, on the other hand, growth in 100 units per cc occurred only with the largest inoculum, and with smaller inocula the amount of penicillin necessary to suppress growth rapidly declined to 1 unit per cc or less.

When the inoculum was grown in the oleic acid-albumin medium, *i. e.*, when it consisted of clumps rather than diffusely growing bacilli, the inhibitory effect of penicillin in the Tween-albumin medium was somewhat less marked. This suggests that the clumps were better able to survive initially in the presence of penicillin. However, the Tween effect was not overcome entirely, possibly because even with clumps as an inoculum, subsequent growth in the Tween-albumin medium was diffuse.

As in the previous experiment, evidence of destruction of penicillin by the tubercle bacil-

lus was slight. Except for the first two tubes in the oleic acid-albumin medium, the end-point was the same whether further penicillin was added or not.

These results were extended to the solid oleic acid-albumin agar medium, on which, even with the smallest inocula (3 or 4 organisms per plate), no inhibition of growth was noted with penicillin in a concentration of 100 units per cc.

In preliminary experiments with contaminated material, quantitative plate counts were made from saline suspensions of the macerated lungs of Swiss mice infected intravenously with H37Rv. The control plates were often so heavily contaminated with other bacteria that the presence or absence of tubercle bacilli could not be determined. Plates containing penicillin in a concentration of 100 units per cc almost invariably showed complete suppression of growth of the contaminants, and in no instance was there evidence of inhibition of growth of the tubercle bacilli.

Comment. The foregoing experiments demonstrate that under appropriate circumstances tubercle bacilli undergo partial lysis in the presence of penicillin. Many other bacteria are lysed by penicillin, some, such as staphylococci, quite rapidly and completely,¹¹ and others, such as anaerobic streptococci, more slowly and to a lesser degree.¹² Tubercle bacilli appear to fall into the latter category.

The explanation for the much greater inhibitory effect of penicillin in the Tween-albumin than in the oleic acid-albumin medium is not entirely clear, but it may be found in part in the more intimate contact between the antibiotic and the individual organisms in the Tween-albumin medium, in which growth is diffuse, than in the oleic acid-albumin medium, in which growth is granular. In addition, specific alterations in the physico-chemical characteristics of the cell, caused by the presence of the non-ionic, surface active wetting agent "Tween 80," may play a major role in the phenomenon. Enhancement of the activity of antimicrobial

¹¹ Kirby, W. M. M., *J. Clin. Invest.*, 1945, **24**, 165.

¹² Todd, E. W., *Lancet*, 1945, **2**, 172.

TABLE II.
Inhibitory Effect of Penicillin on Various Inocula of Tubercle Bacilli in Tween-albumin and Oleic Acid-albumin Media.

Penicillin, units per cc	Inoculum grown in Tween-albumin medium									
	Penicillin added at 3-day intervals					No additional penicillin added				
	Tween medium		Oleic acid medium			Tween medium		Oleic acid medium		
	0	+	0	+	+	0	+	0	+	+
1000	0	0	0	0	0	0	0	0	0	+
100	0	0	0	0	0	0	0	0	0	+
20	+	+	+	+	+	+	+	+	+	+
5	+	+	+	+	+	+	+	+	+	+
1	+	+	+	+	+	+	+	+	+	+
0	+	+	+	+	+	+	+	+	+	+
Inoculum	*1	2	3	4	5	1	2	3	4	5

* 1—2.5-2 mg dry wt. 2—2.5-3 mg dry wt. 3—2.5-4 mg dry wt. 4—2.5-5 mg dry wt. 5—2.5-6 mg dry wt.

agents against other bacteria by various wetting agents is well known.¹³ This increased inhibitory action of penicillin against the tubercle bacillus in the Tween-albumin medium is similar to that recently observed by Fisher with streptomycin.¹⁴

The relatively slight destruction of penicillin by the human virulent strain H37Rv in the present studies is in contrast to the marked destruction reported by others using nonpathogenic laboratory strains.^{2,6} It may be noted that one of these strains, No. 607, which is often used in studies of tubercle bacilli, does not fulfill the cultural and biological requirements of a true tubercle bacillus. It should be stated that since there was relatively little decline in penicillin activity, no attempt was made in the present experiments to determine quantitatively the extent to which the decline was due to inactivation of penicillin by tubercle bacilli, to deterioration in the incubator, or to a secondary outgrowth of resistant variants.

It would appear that the addition of penicillin in concentrations of 50 to 100 units per cc to the liquid or solid oleic acid-albumin medium may prove a valuable adjunct in culturing contaminated materials.

Current methods of treating clinical speci-

mens often do not destroy all of the contaminants, and inhibitory dyes, such as malachite green, which are ordinarily added to the classical egg yolk-potato media, may also inhibit the growth of the tubercle bacillus.¹⁵ It should be realized, of course, that many types of organisms are not inhibited by penicillin, although concentrations as high as 50 to 100 units per cc may be adequate to suppress the growth of many bacteria and fungi not ordinarily considered susceptible.

Summary and Conclusions. Using appropriate experimental conditions, a virulent human strain of *M. tuberculosis* has been found to undergo partial lysis in the presence of high concentrations of penicillin.

Small inocula of tubercle bacilli are highly susceptible to concentrations of penicillin as low as 1 unit per cc in the Tween-albumin medium. In the oleic acid-albumin medium, penicillin in a concentration of 100 units per cc causes no inhibition of growth, even with the smallest inocula.

Preliminary experiments indicate that in both the solid and liquid oleic acid-albumin medium, penicillin in concentrations of 50 to 100 units per cc may prove a valuable adjunct in culturing tubercle bacilli from contaminated materials.

¹³ McCulloch, E. C., *Disinfection and Sterilization*, 2nd Edition, Lea and Febiger, Phila., 1945.

¹⁴ Fisher, M. W., *Am. Rev. Tuberc.*, in press.

¹⁵ Corper, H. J., and Cohn, M. L., *Am. Rev. Tuberc.*, 1946, **53**, 575.

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Some Antibacterial Properties of Mandelamine (Methenamine Mandelate).

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In recent years, the sulfonamide drugs and the antibiotics have dominated the field of chemotherapy. The successful use of these drugs in the treatment of urinary tract infections, however, is dependent upon an unobstructed urine flow plus adequate kidney function. Further, administration of these drugs requires close medical supervision and, finally,

invading organisms tend to develop resistance to both classes of drugs. For these reasons, a relatively non-toxic drug which can be administered by mouth over protracted periods of time to ambulant patients would be highly desirable. According to Carroll and Allen¹

¹ Carroll, G., and Allen, H. N., *J. Urology*, 1946, **55**, 674.