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 specimens 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.

16005

Some Antibacterial Properties of Mandelamine (Methenamine Mandelate).

CHARLES J. DUCA AND JOHN V. SCUDI.

From the Research Laboratories of the Nepera Chemical Co., Inc., Nepera Park, Yonkers, N.Y.

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 Allen[1]

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

TABLE 1.
Stock Laboratory Strains in Normal Sterile Urine Buffered at pH 5.5. Minimal bacteriostatic and bactericidal concentrations of mandelamine, streptomycin, and sulfathiazole given in mg %. Each figure is the average of 5 different series of tests.

	Mandelamine		Streptomycin		Sulfathiazole	
	Static	Cidal	Static	Cidal	Static	Cidal
E. coli 64	50	50	20	20	5	10
" " 4R5190	50	50	20	20	7.5	20
A. aerogenes 129	25	25	20	20	3	5
K. friedlanderi type AF12	12.5	12.5	5	5	5	10
" " " BEGS	25	25	5	5	5	10
St. aureus 209	15	25	10	20	2.5	10
" " SM	20	25	40	40	10	10
Ps. aeruginosa ATCC 9029	40	40	40	40	6.25	20

and Kirwin and Bridges,[2] mandelamine appears to be such a drug. In order to obtain an additional evaluation of the drug, the activities of mandelamine, sulfathiazole and streptomycin against organisms commonly found in urinary tract infections were compared, and the development of resistance by representative organisms to all three drugs was studied. The findings are set forth in the following paragraphs.

Experimental. The medium used throughout this work consisted of normal male urine adjusted to pH 5.5 or 6.5 with phthalate and phosphate buffers in .03 to .06 molar concentrations, respectively. The pH was determined before and after incubation by means of a Coleman model 3 pH electrometer. In the presence of effective bacteriostasis, the pH did not vary by more than 0.5 pH units in any experiment. The inoculum was always 0.1 ml of a 1:5000 dilution of an 18-hr. broth culture. Suitable control tubes of buffered and unbuffered nutrient broth, and of buffered urine were included in each test. If any of the control tubes showed no growth, the whole series was discarded. Eight stock laboratory strains of bacteria commonly found in urinary tract infections were studied at pH 5.5 and 9 strains of organisms recently isolated from urinary tract infections were studied both at pH 5.5 and 6.5. Minimal bacteriostatic concentrations of mandelamine, sulfathiazole and streptomycin were determined by means of a serial 2-fold dilution method. End-points were taken as the lowest drug concentrations at which no growth occurred within 72 hours. Bactericidal activity was determined by subculturing 0.25 ml from each apparently negative tube into 5 ml of nutrient broth followed by examination for visible growth after 72 hours.

Attempts were made to induce resistance in 6 different organisms to streptomycin, sulfathiazole and mandelamine as follows: 0.5 ml of an 18-hour nutrient broth culture was used to inoculate 5 ml of sterile urine, at pH 5.5, in the presence of serial 2-fold dilutions of the individual drugs. After 72 hours incubation, 0.5 ml samples were taken from the tubes containing the highest drug concentrations in which growth had occurred. These were transferred into tubes containing a higher series of drug concentrations, and so on. Finally, when organisms had developed resistance to streptomycin or to sulfathiazole, the antibacterial activity of mandelamine against the resistant organisms was determined.

Results and Discussion. The average minimal bacteriostatic and bactericidal concentrations of sulfathiazole, streptomycin and mandelamine at pH 5.5 against the 8 stock laboratory strains are shown in Table I. The results indicate that minor differences in potency exist but, in general, the activities of all 3 drugs are of the same order of magnitude.

The data obtained at pH 5.5 and 6.5 with the 9 strains recently isolated from urinary tract infections are shown in Table II. The results indicate that streptomycin is somewhat less active at pH 5.5 than at 6.5, the difference being related, presumably, to destruction of

[2] Kirwin, T. J., and Bridges, J. P., *Am. J. Surg.*, 1941, **52**, 477.

† We are indebted to Miss Anna M. Kelly and Miss Mary V. Rothlauf for technical assistance.

TABLE II.

Recently Isolated Strains in Normal Sterile Urine Buffered at pH 5.5 and 6.5. Minimal bacteriostatic and bactericidal concentrations in mg %. Each figure is the average of 5 different series of tests.

pH	Mandelamine				Streptomycin				Sulfathiazole			
	5.5		6.5		5.5		6.5		5.5		6.5	
	static	cidal	static	cidal	static	cidal	static	cidal	static	cidal	static	cidal
E. coli Grogan	25	25	62.5	62.5	10	15	5	10	1.56	5	1.25	20
" " Fernande	50	50	150	150	15	15	5	10	10	20	0.6	0.6
" " Travis	50	50	125	125	20	20	5	5	3	10	1.25	>40
A. aerogenes Harrington	30	30	62.5	62.5	3	5	2.5	2.5	20	20	2.5	40
St. albus No. 6	25	50	75	75	20	20	10	15	.625	2.5	1.25	20
" " Agostini	125	—	75	75	40	—	10	20	5	—	1.25	5
St. hem. No. 9	62.5	—	50	50	20	—	20	20	2.5	—	.6	.6
Ps pyocyaneous No. 7	125	125	200	200	40	40	20	30	5	40	20	40
Ps. aeruginosa Fox	62.5	62.5	200	200	20	20	5	10	2.5	10	2.5	20

TABLE III.

Induced Resistance to Sulfathiazole, Streptomycin, and Mandelamine. Minimal bacteriostatic concentrations of mandelamine before and after development of resistance to sulfathiazole and to streptomycin. Concentrations given in mg %. Each figure is the average of 2 different series of tests.

Organism	Sulfathiazole			Streptomycin			Mandelamine					
	E. coli G	*Aero aero* H	*S. aur.* 209	*E. coli* 64	*Aero aero* 129	*S. aur.* SM	*E. coli* G	*Aero aero* H	*S. aur.* 209	*E. coli* 64	*Aero aero* 129	*S. aur.* SM
Original min. bacteriostatic conc.	20 (*ca.* 75 mg %)	20	20	50	50	15	25	100	200	200	200	20
Final min. bacteriostatic conc.	>Sat.	>Sat.	>Sat.	1500	1200	1500	75	300	75	600	150	20
Increase in resistance	>4X	>4X	>4X	30X	24X	100X	3X	3X	—1.7X	3X	—	—
Number of transfers	5	3	3	8	10	9	9	10	10	9	10	9
Original min. bacteriostatic conc. of mandelamine	25	100	200	200	200	20						
Min. bacteriostatic conc. of mandelamine after induction of resistance	75	300	500	150	75	37.5						

the drug at the lower pH. Sulfathiazole is approximately equally active at both pH levels, but the threat of sulfonamide urolithiasis[3] at the lower level should preclude its use in acidic urine. Mandelamine is more effective at pH 5.5 than at 6.5 but at either pH bactericidal concentrations are low enough to permit ready attainment *in vivo* by appropriate dosing.[1] In connection with this heightened activity at the lower pH values, it is to be noted that mandelamine is an acidifying agent, and Carroll and Allen[1] report, "The urine of all patients (200) in the series but 8 became or remained acid on the therapeutic regimen without other medication or restriction of diet or fluid intake."

As shown in Table III, organisms grew in urine which was saturated, at pH 5.5, with sulfathiazole. The degree of resistance which can be developed is necessarily limited by this restricted solubility of the drug, but within this limitation, a 4-fold increase in resistance occurred after as little as 3 to 5 transfers. Resistance to streptomycin developed more rapidly, an increase of 24- to 100-fold being found after 8 to 10 transfers. In contrast to the foregoing, resistance to mandelamine either did not appear, or if it did, it appeared slowly and only to a limited degree; for example, after 9 to 10 transfers there was no change in the susceptibility of 3 organisms, while with the remaining organisms there was only a 3-fold increase in resistance. From the same table, it appears that sulfathiazole-resistant organisms displayed a slightly increased resistance to mandelamine, while the streptomycin-resistant bacteria did not. Further, it appears that mandelamine retained its bactericidal activity against *A. aerogenes H* and *S. aureus* 209, but was only bacteriostatic to the other 4 organisms. These preliminary findings, which suggest that resistance developed against one drug may alter susceptibility to another chemically unrelated drug, are somewhat anomalous, and are being investigated further.

Summary. A comparison of mandelamine, streptomycin and sulfathiazole against 8 stock laboratory strains and 9 strains of organisms recently isolated from urinary tract infections indicates that their activities are not widely different.

In contrast to the results obtained with streptomycin and sulfathiazole, resistance to mandelamine either did not appear, or if it did, it appeared slowly and to a questionable degree in 3 organisms, while in 3 other organisms, resistance did not appear at all.

Organisms rendered resistant to sulfathiazole or streptomycin remain susceptible to mandelamine.

[3] Scudi, J. V., *Am. J. Med. Sciences*, 1946, **211**, 615.

16006 P

Differences During Dicoumarol Therapy in the Quick and Russell Viper Venom Methods for Prothrombin Determination.*

SLOAN J. WILSON.
(With the technical assistance of LaVonne Coxsey.)
From the Department of Medicine, University of Kansas Medical Center, Kansas City, Kans.

We recently observed a patient with thrombophlebitis and pulmonary emboli who died in a state of hemorrhagic diathesis during dicoumarol therapy. An average daily dose of 240 mg of dicoumarol administered for 14 days had failed to decrease the prothrombin to the desired therapeutic level as measured by the Russell viper venom modification[1,2] of Quick's method.[3] Subsequently the methods

* This study was aided by a grant from the National Research Council.

[1] Fullerton, H. W., *Lancet*, 1940, **2**, 195.