



Description: Many SEATO troops are stationed at various locations throughout Thailand and it was necessary to compare techniques for culture and transport of specimens to ensure the greatest reliability of results. Most specimens were from venereal disease clinic attendants at Korat, Udornthani and Nakornphanom provinces. These attendants were checked weekly and did not necessarily represent suspected causes of gonorrhea. Another group of specimens were from inmates at the Institution for Socially Handicapped Women at Pakret, Nondhaburi province. The remainder of the specimens were from SEATO servicemen.

The venereal disease clinic attendants were interviewed for past and present history of gonococcal infection including treatment and possible re-exposure during treatment. Cervical or urethral specimens were either cultured at the time of sampling or transported to this laboratory in Stuart's Transport medium. Penicillinase was usually added to the plate at the time of streaking. Blood specimens for assay of circulating penicillin were taken from attendants who received an injection of penicillin following pelvic examination. Patients from the Institution for Socially Handicapped Females had been arrested and sent to this institution for rehabilitation. Twenty-six of the 30 subjects chosen were either bacteriologically positive or suspicious for gonococcal infection. The subjects were divided into groups of 5 and six antibiotic regimens were evaluated in terms of blood levels achieved and conversion from positive to negative bacteriological findings.

Initial isolations of N. gonorrhoeae were made on Thayer-Martin medium and sensitivities of isolates to antibiotics were carried out on a solid medium consisting of 4.5% proteose #3, 1.0% hemoglobin, 1.0% Supplement B, and the desired concentration of the antibiotic to be tested. Pure cultures of isolated N. gonorrhoeae were lyophilized for subsequent studies of in vitro sensitivities to antibiotics. The two-fold tube dilution technique was used to assay antibiotic levels of sera. The standard assay organism was the Oxford strain of Staphylococcus aureus. Although it was not planned as a part of this study, the VDRL test was carried out on sera for antibiotic studies. Two-fold dilution tests were carried out on those undiluted sera positive for the VDRL test.

Progress: The use of Stuart's transport medium was disappointing in that positive cultures were obtained from only 7.6 percent of 183 V.D. clinic attendants as compared with 17.2 percent isolates from 437 attendants when cultures were made at the time of sampling. In some instances the time lag from taking the specimen to its arrival at this laboratory was four days which undoubtedly decreased the rate of recovery. Isolations of N. gonorrhoeae from females at Pakret was 48.7 percent and was 60 percent from 25 males in the Bangkok area on Thayer-Martin medium.

Studies were carried out relating positive smears and positive cultures in the diagnosis of gonorrhea. All but 20 of the 465 specimens checked for both smear and culture were from females. There were 29 instances where only the smear was positive; 67 instances where only the culture was positive and 62 instances where both were positive. It was concluded that the culture technique was more

reliable for what in many instances represented chronic rather than acute gonorrhea. Two of the 20 males were positive only for culture and eight for both smear and culture. None had negative cultures and positive smears. It should be emphasized that males were cultured only when they had clinical symptoms of acute infection whereas most specimens from females were taken during weekly routine examinations.

The in vitro sensitivities of N. gonorrhoeae to penicillin G and tetracycline are shown in Table I. (Results are expressed in micrograms/ml rather than units/ml of penicillin G). It was noted that the few strains from males tended to be more resistant than those from females to penicillin G. While many of the 73 strains assayed would be considered penicillin resistant when compared to strains regularly isolated as late as 1955, none were resistant to achievable blood levels of that antibiotic. It was noted that strains from Korat, Udorn, and Pakret were comparable in terms of in vitro sensitivities to penicillin G. Oxytetracycline sensitivities of 27 strains from all four groups ranged from minimum inhibitory concentrations of 0-.4 to 2.2 mcg/ml with a mean and median of 1.2 mcg/ml. Blood levels in excess of these values are easily obtainable with the tetracyclines. There was no indication of cross-resistance between penicillin G and oxytetracycline.

The relationship of penicillin blood concentrations to the minimal inhibitory concentrations for the infecting gonococcus is presumably governed by the time required to effect a bactericidal response at foci of infections. While this time is not known for in vitro circumstances, the time required for in vitro sterilization of "persistors", i.e. the last 0.01 percent of the organisms in a culture, can be as long as 36 hours. Results in Table II show blood concentrations of attendants at the venereal disease clinic in Korat following a single administration of Triplopen, a mixture consisting of 500,000 units of Benethamine penicillin G, 250,000 units of Procaine Penicillin G and 500,000 units of sodium penicillin G. It is evident that for no longer than 8 hours after injection does the median penicillin concentration of blood approximate the median inhibitory concentration of 0.24 mcg/ml determined on isolates from many of these same individuals. A single injection of Triplopen is frequently given as prophylaxis. Evidence for the inadequacy of this prophylaxis was the finding that N. gonorrhoeae was cultured from some individuals within three days following its administration and there were instances of females being named as contacts one or two days after receiving Triplopen.

Six antibiotic regimens were evaluated in the Pakret study. Four regimens included the use of two penicillins now in use by venereologists in Thailand and the other two regimens used a locally available tetracycline derivative reported to enable achievement of very high blood levels. Results in Table III compare the four penicillin regimens used. Regimen A which consisted of 1.2 megaunits of a mixture of benzathine, procaine and potassium penicillin each day for three days was clearly inadequate for treatment of gonorrhea. Blood levels achieved with this antibiotic were comparable to those achieved with Triplopen. The three other

Table II  
 PENICILLIN BLOOD LEVELS FOLLOWING ADMINISTRATION OF TRIPLOPEN \*

Time after Administration	No. of Specimens	Range mcg/ml	Mean mcg/ml	Median mcg/ml
0-4 hour	15	0.1-3.3	1.01	0.67
5-8 hour	27	0.02-3.3	0.3	0.2
9-12 hour	29	0.02-0.33	0.14	0.17
13-16 hour	14	0.01-0.8	0.21	0.18
17-20 hour	7	0.0210.33	0.11	0.08
20-24 hour	21	0.06-0.67	0.14	0.08
2 days	6	0.06-0.67	0.21	0.07
3 days	15	0.06-0.1	0.01	0.06
4 days	15	0.04-0.12	0.02	0.1
5 days	11	0.04-0.1	0.06	0.06
6 days	10	0.06-0.1	0.06	0.06
7 days	6	0.04-0.06	0.05	0.06
8 days	6	0.06-0.1	0.06	0.06
9 days	7	0.04-0.06	0.05	0.06
10 days	2	0.06-0.06	0.06	0.06
11 days	13	0.04-0.06	0.12	0.1
14 days	3	0.06-0.1	0.07	0.06
26 days	2	0.1-0.1	0.1	0.1

\* 1.25 megaunits i.m.

Table I  
 IN VITRO SENSITIVITIES OF *N. GONORRHEA* TO ANTIBIOTICS

Group	Penicillin G			Oxytetracycline		
	MIC* mcg/ml	Frequency distribution	Mean	MIC* mcg/ml	Frequency distribution	Mean
Male patients	0.18	1	0.8	0.4	2	1.2
	0.30	2	1.0	0.8	1	1.24
	0.36	1	1.2	1.0	3	1.2
	0.48	2	1.6	1.6	2	1.2
	0.18-0.6	0.41	0.42	0.8-1.6	1.24	1.2
Udorn (female)	0.06	1	0.4	0.4	2	1.0
	0.12	1	0.8	0.8	1	1.0
	0.18	7	1.0	1.0	3	1.0
	0.24	2	1.2	1.2	2	1.0
	0.06-0.6	0.28	0.24	0.4-1.6	1.0	1.0
Korat (female)	0.30	4	1.4	1.4	2	1.3
	0.36	2	1.6	1.6	1	1.4
	0.48	1	2.2	2.2	1	1.4
	0.5	2	2.2	2.2	1	1.4
	0.03-0.48	0.24	0.18	0.8-2.2	1.3	1.4
Pabret (female)	0.06	1	0.8	0.8	1	1.5
	0.12	1	1.0	1.0	1	1.4
	0.18	2	1.4	1.4	2	1.4
	0.24	3	1.8	1.8	1	1.4
	0.06-0.6	0.30	0.24	0.8-1.6	1.4	1.5
Pabret (female)	0.30	1	1.6	1.6	2	1.5
	0.36	1	2.2	2.2	1	1.5
	0.48	3	2.2	2.2	1	1.5
	0.6	1	2.2	2.2	1	1.5
	0.06-0.6	0.30	0.24	0.8-1.6	1.4	1.5

\*Minimal Inhibitory Concentration

Table III

## LABORATORY FINDINGS OF PAKRET INMATES TREATED WITH PENICILLIN

Regimen	No. of Patients	Time of examination relative to start of therapy							
		Prior		Day 3		Day 8		Day 15	
		Smear	Culture	Smear	Culture	Smear	Culture	Smear	Culture
A	5	5/5	5/5	3/5	3/5	1/5	2/5	2/5	3/5
B	5	2/5	4/5	1/5	0/5	0/5	0/5	0/3	1/3
C	5	2/5	3/5	0/5	0/5	0/5	0/5	0/4	0/4
D	5	3/5	2/5	0/5	0/5	0/5	0/5	0/3	0/3

Regimen A: Consisted of Penadur 1.2 Megaunits IM per day for 3 days.

Regimen B: Consisted of Penadur 2.4 Meganuits IM per day for 3 days.

Regimen C: Consisted of Penadur 1.2 Megaunits + Triplopen 1.25 Megaunits IM per day for 3 days.

Regimen D: Consisted of Triplopen 2.5 Megaunits IM per day for 3 days

Penadur: 1.2 megaunits contained 6000,000 units benzathine penicillin G.  
300,000 units procaine penicillin G.  
300,000 units potassium penicillin G.

Triplopen: 1.25 megaunits contained 500,000 units benethaminepenicillin G.  
250,000 units Procaine penicillin G.  
500,000 units Sodium penicillin G.

Table IV

## BLOOD LEVELS OF PENICILLIN IN PAKRET INMATES

Regimen*	No. of Patients	Average Penicillin Blood Levels (mcg/ml)at										
		0 Hrs	4 Hrs	23 Hrs	28 Hrs	47 Hrs	51 Hrs	3 Days	4 Days	5 Days	6 Days	7 Days
A	5	0.2	1.7	0.15	1.4	0.23	1.16	0.44	0.12	0.2	0.2	0.2
B	5	0.2	4.65	1.04	3.97	0.62	4.65	1.34	0.9	0.1	0.14	0.16
C	5	0.2	3.65	0.9	4.15	0.82	4.82	1.08	1.16	0.24	0.18	0.28
D	5	0.2	5.3	1.0	3.15	0.96	3.66	1.84	1.34	0.18	0.20	0.28

\* See Table III for detailed description of regimens.

regimens consisted of approximately double the dosage of regimen A. Bacteriologically all of the 11 patients positive initially became negative but one patient in the regimen B group was positive when sampled on the 15th day. This one was considered a relapse because confinement of inmates decreased the likelihood of reinfection. Penicillin blood levels of regimens A-D are shown in Table IV. Antibiotics were administered at 0, 24 and 48 hours. Statistical analyses of these data showed no significant differences among groups B, C and D but penicillin blood levels of all three were significantly higher than that noted with regimen A. Of greater importance was the fact that the average penicillinemia of groups B, C and D remained higher for 4 days than the minimal inhibitory concentrations of any strain of N. gonorrhoeae isolated in Thailand during this study. It is evident from the results in Table III that 96 hours of sustained blood levels in excess of the minimal inhibitory concentration of the organisms was sufficient to effect clinical cures in 10 of 11 subjects.

Laboratory findings of Pakret inmates treated with tetracycline are shown in Table V. Both regimens were considered therapeutic successes in that all of the eight positive cases were negative on the day 20. Blood levels of tetracycline, shown in Table VI, were consistently high in the group receiving 2.0 gm/day. Tetracycline levels of the group receiving 1.0 gm/day was rarely measured higher than the minimal inhibitory concentration of 1.2 mcg/ml. While the blood levels of tetracycline were considerably lower than reported by other investigators with using tetracycline bitartrate, the levels achieved compared favorably with other oral forms of tetracyclines. The dosage of 2.0 gm/day for 5 days was probably higher than the optimum dosage of the drug. Additional "titration" of the dosage will be necessary to determine the least amount of drug that will produce an effective clinical response and be acceptable from an economic point of view.

Results of the VDRL screening tests for possible syphilis are shown in Table VII. The danger of misinterpretation of results of the VDRL test i.e. false positive reactions from individuals with leprosy, malaria, tuberculosis, hepatitis, etc., is recognized. However its value as a screening test is almost undisputed when used in conjunction with epidemiological investigations. Reactive results in contacts, suspects or associates are significant especially when reactive in high or rapidly increasing titer. The finding that 94 of 260 attendants of venereal disease centers and inmates at Pakret were positive, many of them at high titers, is indicative of a reservoir of syphilis. Twelve of 81 American servicemen were positive at low titers. The paucity of clinical syphilis reported in American servicemen is thought to be related to administration of spirocheticidal drugs administered for a variety of reasons but having as a side effect the suppression of undetected active or latent syphilis.

Preliminary findings from the study attempting to relate penicillinase-producing Staphylococcus aureus as a factor in penicillin-refractory cases of gonorrhea are presented in Table VIII. Only 8 of the 172 patients had both organisms present on culture. All were coagulase-positive and penicillin-resistant. Their capacity for producing penicillinase has not been determined. Future studies on this aspect

Table V

## LABORATORY FINDINGS OF PAKRET INMATES TREATED WITH ORAL TETRACYCLINE\*

Regimen	No. of Patient	Time of Examination Relative to Start of Therapy							
		Prior		Day 2		Day 6		Day 20	
		Smear	Culture	Smear	Culture	Smear	Culture	Smear	Culture
E	N 1	+	+	-	-	-	-	-	-
	N 7	+	-	+	-	-	-	-	-
	N 8	-	-	-	-	-	-	-	-
	N 23	-	-	-	-	-	-	-	-
	N 28	+	+	-	-	-	-	-	-
F	N 16	+	-	+	-	-	-	-	-
	N 19	+	+	-	-	-	-	-	-
	N 31	+	+	-	-	-	-	-	-
	N 32	+	-	-	-	+	0	-	-
	N 33	+	-	-	-	-	-	-	-

+ Positive  
 - Negative  
 0 Not done

\* Tetracycline bitartrate is produced by N.V. Minerva-Chemie under the trade name of "Mervacycline"

Regimen E: Consisted of oral administration of 250 mg at 0900, 13.00, 17.00 and 21.00 hours each day for 5 days.

Regimen F: Consisted of oral administration of 500 mg at 09.00, 13.00, 17.00 and 21.00 hours each day for 5 days.

Table VI

## BLOOD LEVELS OF TETRACYCLINE IN PAKRET INMATES

Regimen*	Before treatment	Blood Concentrations of Tetracycline (mcg/ml) at								
		0 Hrs	3-3.5 Hrs	5-5.5 Hrs	23.5-24 Hrs	26.5-29 Hrs	44-46 Hrs	48-50.5 Hrs	66.5-70 Hrs	100-124 Hrs
E										
Average	< 0.2	0.48	0.52	1.04	1.78	0.96	1.6	1.6	0.44	
F										
Average	< 0.2	1.9	1.82	1.64	2.14	3.06	3.64	2.12	2.18	

Regimen See Table V

Table VII  
VDRL Analyses in Thailand (January - March 1965)

Source of Specimens	Total Specimens Tested	Total Negative	Total Positive at								
			undil.	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256
<u>Thais</u>											
Nakornpanom	30	25	1	1	-	-	1	-	1	1	-
Pakret	60	31	10	5	6	4	-	2	-	1	1
Udorn	30	22	3	3	-	1	1	-	-	-	-
Korat	133	82	13	14	12	8	2	2	-	-	-
Miscellaneous	7	6	1	-	-	-	-	-	-	-	-
<u>Thai Totals</u>	260	166	28	23	18	13	4	4	1	2	1
<u>Americans</u>											
Nakornpanom	89	78	8	3	-	-	-	-	-	-	-
Miscellaneous	4	3	-	-	1	-	-	-	-	-	-
<u>American Totals</u>	93	81	8	3	1	-	-	-	-	-	-

Table VIII  
ISOLATIONS OF STAPHYLOCOCCI AND GONOCOCCI FROM CERVICAL OR URETHRAL SWABS

Total Cultured	Sex	Staph + G-C +	Staph - G-C +	Staph + G-C -	Staph - G-C -
160	F	6	24	65	65
12	M	2	0	5	5

will consist of culturing only penicillin-refractory cases and attempting to demonstrate penicillinase in urethral discharges. It should be noted that detailed documentation of penicillin-refractory gonorrhea among American servicemen is difficult to find.

Summary: The use of Stuart's transport medium for shipment of specimens to Bangkok was not satisfactory. Direct streaking onto Thayer-Martin medium and incubation in a candle jar at 37 C for 24 hours prior to shipment to Bangkok was considered the method of choice. In vitro sensitivities of N. gonorrhoeae to penicillin revealed that all 73 strains tested were sensitive to 1.0 unit (0.6 mcg)/ml of penicillin G. Oxytetracycline sensitivities of the 27 strains tested ranged from 0.4 mcg/ml to 2.2 mcg/ml with a mean and median of 1.2 mcg/ml. Measurement of penicillinemia of attendants at venereal disease control centers indicated that injection of a single vial of Triplopen failed to provide blood levels greater than the median MIC of isolates of N. gonorrhoeae for more than 8 hours. Six antibiotic regimens were evaluated in 30 inmates of the Institute for Socially Handicapped Females. Data based on blood level studies and conversion from positive to negative cultures indicated that 2 vials of Triplopen, Penadur, or 1 vial of each administered daily for 3 days was therapeutically successful, but one vial of Penadur daily for 3 days was inadequate. Dosages of 1 gm/day or 2 gm/day for 5 days of oral tetracycline bitartrate were effective therapeutically but blood levels of tetracycline were, at best, minimal with the lower dose. About 36 percent of 260 sera, mostly from attendants of F.C. control centers, and 12 of 93 sera from American were VDRL positive. Eight of 32 specimens positive for N. gonorrhoeae were simultaneously positive for S. aureus.

Conclusion: One of the causes of the high prevalence of gonorrhea in Thailand is inadequate treatment of N. gonorrhoeae strains which have become more resistant to penicillin in recent years. It is postulated that in that blood levels of penicillin were frequently less than the MIC of the causal organism when currently recommended therapeutic regimens of penicillin were used. The use of a single vial of Triplopen for prophylaxis of gonorrhea in individuals at high risk is probably inadequate. Emphasis in future studies will be (1) evaluation of antibiotic prophylaxis in the prevention of gonorrhea (2) further evaluations of antibiotic regimens and (3) attempts to demonstrate penicillinase in urethral exudates.

General Information: During the period covered by this report 1845 routine specimens processed were as follows:

Water samples	773
Stool cultures	597
Urine specimens	93
Throat swabs	52
Pus specimens	15
Sputum specimens	10
Blood cultures	10
Miscellaneous cultures	32

Sera for

VDRL Test	161
Widal Test	34
Febrile Agglutination Test	39
Heterophile Test	<u>29</u>

Total 1,845  
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An additional 626 cultures were made in support of the special study on Thai Hemorrhagic Fever study carried out in July, August and September of 1964.

The following organisms were added to the SEATO Medical Research Laboratory culture collection during the past year:

<u>Organisms</u>	<u>Strains</u>	<u>Organism</u>	<u>Strains</u>
Salmonella paratyphi A	1	Shigella boydii 1	1
Salmonella paratyphi B	16	Shigella boydii 2	2
Salmonella derby	23	Shigella boydii 4	1
Salmonella sainpaul	2	Shigella boydii 5	1
Salmonella sandiego	1	Shigella boydii 7	5
Salmonella stanley	5	Shigella dysenteriae 1	5
Salmonella typhimurium	3	Shigella dysenteriae 2	3
Salmonella heidelberg	1	Shigella dysenteriae 3	2
Salmonella schelessheim	1	Shigella dysenteriae 4	1
Salmonella montevideo	9	Shigella dysenteriae 6	1
Salmonella oslo	5	Shigella flexneri 1	1
Salmonella tennessee	1	Shigella flexneri 2	4
Salmonella potsdams	1	Shigella flexneri 3	5
Salmonella cholerae suis	1	Shigella flexneri 4	4
Salmonella virchow	2	Shigella flexneri 6	3
Salmonella bovismorbificans	3	Shigella sonnei form 1	8
Salmonella newport	3	Shigella sonnei form 2	12

<u>Organisms</u>	<u>Strains</u>	<u>Organisms</u>	<u>Strains</u>
Salmonella burgedorf	2	Shigella alkalescens-dispar 01	5
Salmonella dublin	2	Shigella alkalescens-dispar 04	1
Salmonella mendoxa	1		
Salmonella panama	1		
Salmonella typhosa	9		
Salmonella seremban	1		
Salmonella enteritidis	1		
Salmonella give	1		
Salmonella lexington	11		
Salmonella muenster	1		
Salmonella meleagridis	3		
Salmonella weltevreden	6		
Salmonella anatum	2		
Salmonella seftenberg	1		
Salmonella welikada	1		