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CLINICAL AND LABORATORY STUDIES OF THE EPIDEMIOLOGY,  
ETIOLOGY, AND TREATMENT OF URETHRITIS  
IN THE MALE

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In pursuing these graduate studies, I have become deeply indebted to LCDR David Johnson, whose patience and understanding were limitless.

## PREFACE

Gonorrhoea is one of the most common bacterial diseases of man. In 1966, an estimated 1,500,000 people in the United States acquired gonorrhoea. During periods of military conflict, this disease becomes extraordinarily common in military personnel. For example, over 66,000 cases of gonococcal urethritis occurred among U. S. Forces in Viet Nam in 1966. In some areas, the annual incidence of gonorrhoea approached 500 cases per 1,000 men. The second most common disease of military personnel is nongonococcal urethritis, a general classification of those urethritides which are not associated with demonstrable N. gonorrhoeae, but may not have a single etiology. Taken together, gonococcal and nongonococcal urethritis are several times as common as any other reportable infectious disease in military populations in the Far East.

The series of studies from which this dissertation is drawn began early in 1965, and is still continuing. The studies were generated by the alarming increase in incidence of urethritis accompanying the Viet Nam build-up; by the unexpectedly high failure rate accompanying standard penicillin therapy of gonorrhoea; by the lack of a consistent approach by military physicians to the diagnosis and treatment of nongonococcal urethritis; and by the immediate need for a practical solution to these problems. This research was approved and undertaken for the expressed purpose of finding an adequate treatment for urethritis; and of effecting preventive measures to reduce the incidence of urethritis in military personnel. However, the philosophy of the investigators was that these studies also afforded an opportunity to learn more about the epidemiology,

etiology, and pathogenesis of the various urethritides.

As the investigation progressed, a "story" became apparent which tied together gonorrhea, postgonococcal urethritis, and nongonococcal urethritis as sequential stages in the development of chronic urethritis in certain situations. It seemed appropriate, therefore, to describe this "story" in a series of manuscripts, dealing with certain aspects of the overall problem of urethritis, but presented in an order which corresponds to the natural sequence with which chronic urethritis often develops in the male. These manuscripts are included as chapters in this dissertation, in the same order in which they were prepared for publication. Chapter three is not yet finished. It is partly based upon recently completed work with gonorrhea infection in women, but is inserted into the dissertation since it pertains to the epidemiology of gonococcal infection in men.

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CHAPTER I  
STUDIES OF VENEREAL DISEASE. I. PROBENECID-  
PENICILLIN COMBINATION AND TETRACYCLINE IN THE  
TREATMENT OF "PENICILLIN-RESISTANT" GONORRHEA IN THE MALE

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authors and are not to be construed as official or reflecting the  
views of the Navy Department or the Naval Service at large.

## Synopsis-Abstract

Treatment of gonorrhea in men with a single intramuscular dose of 2.4 megaunits of procaine penicillin G (PPG), recommended by the USPHS and the Armed Forces in 1965, continues to result in 20-30% treatment failures among military personnel in the Far East. In studies of confined populations aboard aircraft carriers, 63 men with gonorrhea received 2.4 megaunits of PPG with 18 (29%) treatment failures; 58 men received 2.4 megaunits PPG plus oral probenecid, with only one failure. Resistance to .06 units of penicillin/ml media was noted in 66% and 77% of N. gonorrhoeae isolated from the respective groups. Thirty men received oral tetracycline, with no failures. In areas where treatment of gonorrhea with large doses of parenteral PPG is becoming less effective, adequate therapy is possible with probenecid plus PPG or with tetracycline.

Keywords: Gonorrhea  
Procaine Penicillin G  
Probenecid  
Tetracycline

The world-wide emergence of strains of Neisseria gonorrhoeae with decreased sensitivity to penicillin has been associated with a growing number of therapeutic failures when acute gonococcal urethritis in males is treated with doses of penicillin previously considered adequate.<sup>1</sup> Although these failures have been ascribed variously to the associated presence of penicillinase-producing staphylococci,<sup>2</sup> or to diagnostic confusion in cases of urethritis caused by organisms of the ill-defined "Tribe Mimea,"<sup>3</sup> it appears likely that the great majority of failures are a result of lessened penicillin sensitivity of gonococci. Recent reports have shown that most treatment failures are limited to urethritis caused by penicillin-resistant gonococci, and that this relative resistance can be overcome by using higher doses of penicillin.<sup>4</sup> Current clinical experience suggests that a single, large dose of procaine penicillin is more effective than repeated smaller doses; is also better than combinations of intermediate and long acting penicillin in comparable doses; and is far better than long acting penicillin alone, such as bicillin, which should not be used in gonorrhea treatment,<sup>5,6</sup>

In view of this experience, the U. S. Public Health Service and the Armed Forces, in the summer of 1965, recommended the use of a single intramuscular dose of 2.4 million units of aqueous procaine penicillin for the treatment of male gonococcal urethritis.<sup>7,8</sup>

In the intervening period since the widespread adoption of this treatment schedule, it has become apparent, through routine surveillance of communicable disease reports from military medical facilities, that an appreciable treatment failure rate continues to exist in Southeast Asia and the Western Pacific. For example, in a study of the

effectiveness of penicillin in the treatment of gonorrhoea in Korea, 3,270 cases were treated with the recommended dose of 2.4 million units of aqueous procaine penicillin.<sup>9</sup> There were 642 failures (19.6%). Seven of the failures were lost from the study and 635 were retreated with 4.8 million units of procaine penicillin. One hundred and eight (17%) failed to respond to the larger dose.

In an effort to assess further the efficacy of the above treatment in an area where strains of Neisseria gonorrhoeae with lessened penicillin sensitivity are common, the first phase of the present study was designed to utilize a group of men who would be exposed to gonorrhoea over a short period of time and then be removed from chance of reinfection until after treatment and follow-up studies were completed. A crew of a Navy ship given "liberty" for a few days in Subic Bay in the Philippines provided such a group. Venereal disease rates are high among service personnel in this area and a previous survey (1961) had shown that 100% of gonococcal isolates demonstrated lessened sensitivity to penicillin.<sup>10</sup>

When a failure rate of nearly 30% was encountered among this first group of men (as presented later under "Results"), it was apparent that a more effective treatment should be sought. Although some consideration was given to evaluating a single intramuscular dose of 4.8 million units of procaine penicillin, this idea was rejected, since the experience in Korea, as well as that of the authors, indicated that a certain percentage of cases would not respond to this dose. Furthermore, the volume of penicillin emulsion required was so large (4.8 million units of procaine penicillin G is commercially available in injectable volumes

of eight to sixteen ml) that further increases of gonococcal penicillin resistance in the future could not be practically met by further increases in dosage of parenteral procaine penicillin.

Since the serum level of penicillin can be increased from two to twenty-fold, and its serum half-life prolonged markedly, by concurrent administration of probenecid,<sup>11</sup> it was reasoned that probenecid might extend the usefulness of penicillin in the treatment of gonorrhoea. Therefore, it was decided to compare the results of combined therapy of probenecid and 2.4 million units of procaine penicillin with the results that had been achieved with the same dosage of procaine penicillin alone.

Conditions of this study also appeared to be opportune for further evaluation of tetracycline in the treatment of gonorrhoea. This antibiotic, and its congeners, have been used successfully against "penicillin-resistant" gonorrhoea in the male by others.<sup>12</sup> However, some of the previous studies have been weakened because of relatively short follow-up periods, or because gonococcal cultures were not used in diagnosis. Additionally, in most studies the patients have been relied upon to administer the tetracycline to themselves, and the possibility of reinfection was not completely eliminated. Furthermore, regional differences in the efficacy of the drug might be expected since increased resistance to tetracycline has been found in 49% of gonococcal isolates from the Philippine Islands but is less common elsewhere.

#### Materials and Methods

Study Population.--The evaluation of one dose of 2.4 million units of

procaine penicillin alone in the treatment of gonorrhea was carried out aboard an aircraft carrier with a crew of about 3000 men. After 55 consecutive days at sea this ship visited Subic Bay, Philippine Islands, for five days, followed immediately by another five days of liberty in Hong Kong, and then put out to sea for eight weeks. The investigating team boarded the carrier in Subic Bay in early November 1965 and remained aboard for three weeks. All patients who reported to sick call with symptoms of urethritis and a history of recent sexual exposure were evaluated, and those who were diagnosed as having gonorrhea were included in the study group.

In the second phase of the study, the evaluation of probenecid plus 2.4 million units of penicillin and of tetracycline in the treatment of gonorrhea was carried out aboard a larger carrier with a crew of about 5,500 men. This ship had been at sea for 33 consecutive days before entering Subic Bay for six days of liberty and then returned to sea for 30 more consecutive days. The investigating team boarded the ship in mid-April, 1966, at Subic Bay and remained aboard for five weeks. Again all patients who presented themselves with symptoms of urethritis and were diagnosed as having gonorrhea were included in the study population and followed until the ship returned to port. Those with non-gonococcal urethritis (NGU) were also carefully evaluated and followed. Treatment of NGU was with either tetracycline or a placebo under a double blind system. The results, which clearly favored tetracycline, will be reported separately.

Initial Evaluation.--All patients were interviewed extensively regarding past venereal disease, sexual exposure, recent antibiotic treatment, symptoms, and basic demographic information, utilizing standardized forms. Loopsful of exudate were obtained from patients presenting a gross discharge by passing a 26 gauge platinum loop one inch into the urethra. These were immediately examined by Gram stained smears and were cultured for gonococci and certain other bacteria. When a presumptive diagnosis of gonorrhoea could be made from the initial stained smear of urethral exudate treatment was started without delay. Patients with a Gram stain negative for gonococci were required to return after 24 hours. If the previous day's cultures for gonococci were negative, the Gram stain and cultures were repeated. If negative results were obtained from these, a third Gram stain and cultures were obtained. A diagnosis of NGU was then made if all studies failed to reveal gonococci.

Patients without gross discharge were evaluated three times by the same schedule, except that a two-glass urinalysis was also performed, and the sediment of the first morning urine was cultured each time for gonococci and other bacteria.

Laboratory Methods.--Loopsful of exudate or of urine sediment were cultured as follows:

- (1) For the isolation of gonococci two different media were employed for each specimen: Mueller-Hinton agar, to which 4% human blood was added and chocolate, followed by the addition of Bacto Supplement B and Ristocetin-Polymyxin B and adjustment of pH to 7.4;



and Todd-Hewitt broth-blood agar plus the same additives at the same pH. Cultures were incubated at 37°C in candle jars for 24 to 48 hours before examination. All gonococcal isolates were confirmed by microscopy, oxidase tests, and reactions on dextrose, maltose, and sucrose fermentation plates. To determine sensitivity to penicillin, gonococcal strains, carried on chocolate agar plates, were inoculated heavily in single streak lines onto Mueller-Hinton agar plates containing crystalline penicillin G in concentrations of 0.06, 0.125, 0.250, 0.500 and 1.00 International Units per ml of media and also onto control plates containing no antibiotic.

(2) Plain human blood agar and MacConkey agar were inoculated with exudate and incubated aerobically in an attempt to isolate penicillinase-producing staphylococci and the Gram negative cocco-bacillary organisms which have sometimes been taxonomically placed in the Tribe Mimea.

Gonorrhoea Treatment Programs.--Three antibiotic regimens were evaluated:

(1) Procaine penicillin G in a single intramuscular dose of 2.4 million units, given as 1.2 million units in each hip, was administered to all patients with gonorrhoea in the first study.

(2) The same dose of procaine penicillin G in combination with probenecid was administered to two-thirds of the men with gonorrhoea in the second study. One gram of probenecid was given orally one hour prior to the penicillin injection, and 500 mg were given 6, 12 and 18 hours after the injection.

(3) Tetracycline was administered to every third patient found to have gonorrhoea during the second study. The oral dosage was 1500 mg

(six 250 mg tablets) initially, followed by 500 mg every six hours for sixteen doses.

All oral medications were personally dispensed next to a water fountain by a member of the investigating team. Each patient was watched while he swallowed the tablets, and patients were frequently observed to ensure that pills had been swallowed and not concealed in the mouth.

Follow-up.--Patients were reexamined at five-day intervals after treatment. Two-glass urinalyses were obtained at each revisit, cultures for gonococci and other bacteria were done when symptoms or discharge recurred or persisted, and were repeated in conjunction with rectal examinations and prostatic massage when routine cultures were negative in the face of suspected recurrences. The median follow-up was ten days in the first study, with a range of five to twenty days. During the second study only three patients were followed for less than 20 days, most were followed for 25 to 30 days, and there were no absentees from over 400 scheduled reexaminations.

Duration of Gonococcal Viability after Initiation of Treatment.--In addition to the routine follow-up, exudates from the first 28 patients in the second study were examined microscopically and culturally at two-hourly intervals for twelve hours, and then again at 24 hours, after treatment was started. This was done to compare the duration of infectivity following administration of penicillin and of tetracycline.

## Results

Comparison of Three Treatment Schedules.--On the first aircraft carrier 119 cases of urethritis were studied. Gram stains of urethral exudate from 72 of these patients showed typical gonococci, and Neisseria gonorrhoeae were cultured from 68 of the 72. Sixty-three of the cases proven by culture were treated with a single injection of 2.4 million units of procaine penicillin G. As shown in Table 1, gonococci were not eliminated from 18 patients. Fourteen of these treatment failures were retreated with a single intramuscular dose of 4.8 million units of procaine penicillin G and three remained infected, as determined after five days follow-up.

On the second carrier 194 patients with acute urethritis were studied and 88 cases of gonorrhea were found. There was 100% correlation between the microscopic and cultural identification of Neisseria gonorrhoeae in all 88 cases. Table 1 shows that 58 cases were treated with penicillin-probenecid with only one treatment failure. This single failure was remarkable in having had a greater number of organisms per oil immersion field on his initial Gram stained smear than any other patient seen in both studies. He was retreated successfully with 1.2 million units of procaine penicillin G, intramuscularly, every twelve hours for eight doses, plus 500 mg of probenecid orally every six hours for 16 doses.

Thirty cases were treated with tetracycline with no failures.

Penicillin Sensitivity of Gonococcal Isolates.--Evidence of significantly decreased sensitivity to penicillin among the strains of

N. gonorrhoeae recovered during both studies is presented in Table 2.

Sixty-three per cent of the strains from the first study and 77% of those from the second were able to grow in the presence of 0.06 units per ml of media, whereas none of the strains encountered by various authors prior to 1954 had shown resistance to 0.05 units per ml.<sup>1</sup> Although the results obtained by the plate dilution method depend on the inoculum and the type of medium used,<sup>13</sup> the association of treatment failures with gonococcal resistance to 0.06 units or more of penicillin per ml of media in the present study suggest that resistance at this level was of clinical significance. In the first study, the penicillin sensitivities of the initial gonococcal isolates were determined for 17 of the treatment failures. Seven were inhibited by 0.06 units of penicillin per ml and ten were not. However, penicillin sensitivity testing was repeated on six strains recovered after treatment, and all were resistant to 0.125 units or more of penicillin per ml. Five of these six resistant strains were recovered from patients who prior to treatment had harbored gonococci which were inhibited by 0.06 units per ml. Thus, the failure to respond to penicillin was due either to infection with strains of N. gonorrhoeae which were initially resistant to 0.06 units of penicillin per ml, or to the selection of strains with increased penicillin resistance as a direct result of penicillin treatment.

Duration of Gonococcal Viability after Initiation of Treatment.-- Viability after treatment with penicillin-probenecid or with tetracycline, as determined by recovery of the organisms from urethral exudate, is shown in Figure 1. Among 20 patients receiving penicillin-probenecid, only five continued to harbor live organisms after six

hours of treatment. At the end of nine hours only one was excreting live gonococci. This patient was the sole treatment failure in the penicillin-probenecid group and he continued to have viable urethral gonococci until the second day of his retreatment.

Among the patients receiving tetracycline, viable gonococci were recovered from all eight after six hours, from six after nine hours and from two patients at twelve hours. Although the last two patients were not subsequently recultured, very few viable organisms were recovered from either at twelve hours.

Presence of Mimeo and Penicillinase-Producing Staphylococci.-- Cultures from 119 patients during the first phase of the study and from 182 during the second yielded only eight strains of organisms with some of the morphological and biochemical characteristics of the so-called Tribe Mimeo. All eight strains were oxidase-negative and failed to ferment dextrose, maltose, and sucrose. Further identification could not be carried out in the field.

Penicillinase-producing staphylococcal strains which permitted satellite growth of susceptible N. gonorrhoeae on media containing penicillin were recovered during both studies, but never in association with a treatment failure.

Adverse Drug Reactions.--Although no specific inquiries were made about the occurrence of side effects, patients were observed every six hours during the course of treatment and at 5 day intervals thereafter. The use of 2.4 million units of penicillin alone was associated with no ill effects. One mild urticarial reaction began within two hours after starting treatment with penicillin-probenecid and was rapidly and completely suppressed by giving an antihistamine for four days. One

patient vomited shortly after receiving 1.5 grams of tetracycline, but had no further trouble when the same dose was readministered soon afterwards. Another patient complained of loose stools on the third day of tetracycline but this did not necessitate discontinuation of treatment. After completing a course of tetracycline, one patient developed a pruritic, maculopapular rash in the anticubital fossae which slowly resolved over ten days.

Incidence of PGU.--Post gonococcal urethritis occurred significantly more often after penicillin-probenecid than after tetracycline. These results are described separately.<sup>14</sup>

#### Discussion

Treatment of Penicillin-Resistant Gonorrhoea.--The proportion of strains of gonococci which have acquired relative resistance to penicillin is increasing throughout the world.<sup>1,13</sup> In Olongapo, the Philippine town outside the gates of the Subic Bay Naval Base where the gonorrhoea covered by this report was acquired, penicillin resistance can perhaps be attributed to the ill-advised use of noncurative doses of procaine penicillin G or of benzathene penicillin G in infected females. It is interesting to speculate on how much more penicillin resistance will be encountered in the future. Gonococcal resistance to as much as 21 units of penicillin per ml of media has been produced by in vitro manipulations,<sup>15</sup> but this is still only a relative resistance in contrast to the absolute resistance to streptomycin which may be found in gonococcal isolates.<sup>10</sup>

Thus, in the future, penicillin dosage theoretically could be increased as needed to meet further increases in gonococcal resistance.

It has been estimated that penicillin serum levels of ten times the minimal inhibitory concentration of the infecting strains of the organisms are required to effect cure of gonorrhoea.<sup>4</sup> Because of the great individual and sex differences in serum levels resulting from a given injected dose of penicillin, a universally effective dose would have to be large enough to achieve these high serum levels in everyone. As previously stated, it is apparently more important to attain a very high serum level of penicillin for a short time than to reach levels which are marginally inhibitory for longer periods. An analogous finding<sup>16</sup> is that most gonococci are killed within 24 hours by in vitro exposure to bacteriocidal levels of penicillin, with none surviving after 48 hours; whereas gonococci exposed to lower, bacteriostatic doses for prolonged periods remain viable and recover when penicillin is removed.

Use of Probenecid.--In recent years increased penicillin resistance generally has been dealt with successfully by progressively increased single doses of short and intermediate acting penicillin. From the practical standpoint, however, there is a limit to the amount of penicillin that can be injected by the intramuscular route. Fortunately, the time-action curve of penicillin can be affected by decreasing the rate of excretion of the antibiotic with probenecid. Renal tubular secretion of penicillin can be continuously inhibited in the adult by the administration of oral probenecid in a dosage of 500 mg every six hours.<sup>11</sup> The use of this adjunctive agent in the treatment of gonorrhoea is not without precedent. Jacoby and co-workers,<sup>17</sup> in 1954, had observed a 25% failure rate in 137 gonorrhoea patients treated with 300,000 units

of oral penicillin G, in comparison to a 12% failure rate in 115 patients treated with the same dosage of penicillin plus one gram of probenecid. In the present study the effectiveness of oral probenecid in potentiating intramuscular procaine penicillin G in the treatment of male gonorrhea has been demonstrated. The importance of high blood levels of penicillin rather than high urine levels can be inferred from both studies.

The administration of the first dose of probenecid one hour before the penicillin in the present study was based on Boger's studies,<sup>11</sup> which showed that effective plasma levels of probenecid are attained one hour after oral ingestion of one gram. Since the serum half life is six to twelve hours, the three subsequent doses of 500 mg of probenecid at six hour intervals were sufficient to maintain maximal inhibition of renal tubular secretion of penicillin for over 24 hours.

Probenecid has been used in the treatment of gout for 15 years, and appears to be a relatively safe drug. The principal side effects are gastrointestinal disturbances and hypersensitivity.<sup>18</sup> The main disadvantage to using it in combination with penicillin is inherent in any combined therapy. When an allergic reaction occurs, it is often difficult to determine which drug is responsible for the allergy. This situation arose only once in the present series of 58 patients treated with penicillin-probenecid.

Use of Tetracycline.--Although a 1961 WHO survey<sup>10</sup> showed that 49% of the strains of *N. gonorrhoeae* isolated in the Philippines have lessened sensitivity to tetracycline, there were no treatment failures following tetracycline therapy during the present study. Thus it would appear to be an excellent drug for the treatment of gonorrhea. However, from the public health standpoint, it is more expensive than penicillin



and has all the disadvantages of a prolonged, multiple dose regime requiring self-administration of the drug. Although tetracycline congeners have been given as a single oral dose with some success in the past, Sokoloff<sup>12</sup> found that a single oral dose of 900 mg of dimethylchlorotetracycline, the equivalent of 1500 mg of tetracycline, resulted in a 36% failure rate in gonococcal urethritis in males. His results demonstrated the necessity of continuing treatment for at least three days, and preferably for five days. It was the experience of this study that four days are adequate, and tetracycline, in the dosage employed, can be recommended as an effective alternative to penicillin-probenecid in the treatment of acute gonococcal urethritis in males. It is possible that single oral doses of tetracycline in excess of 1500 mg could obviate the necessity for continuing therapy for four days, but this remains to be proven.

In areas where gonococcal penicillin resistance is a problem, a practical approach to the treatment of male gonorrhoea can be formulated on the basis of the above findings. Initial treatment with either penicillin-probenecid or with tetracycline according to the schedules used in this study is effective in eradicating N. gonorrhoeae from the urethra. The former regimen has the disadvantage of a higher incidence of postgonococcal urethritis, while the latter has the disadvantage of placing the responsibility for antibiotic administration to the hands of the patient.

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Generic and Trade Names of Drug

Probenecid - Benemid

Table 1. A comparison of treatment failures following three antibiotic schedules in acute gonococcal urethritis in males

Treatment Schedule	Number Cases	Number Failures	% Failures
Procaine penicillin G, 2.4 million units in a single IM dose	63	18	29
Procaine penicillin G, 2.4 million units IM, plus oral probenecid*	58	1	2
Tetracycline, 1.5 gm orally, followed by 500 mg q6h x 4 days	30	0	0

\*Probenecid was administered orally as follows: 1000 mg one hour before injection of penicillin and 500 mg at 6, 12, and 18 hours after injection.

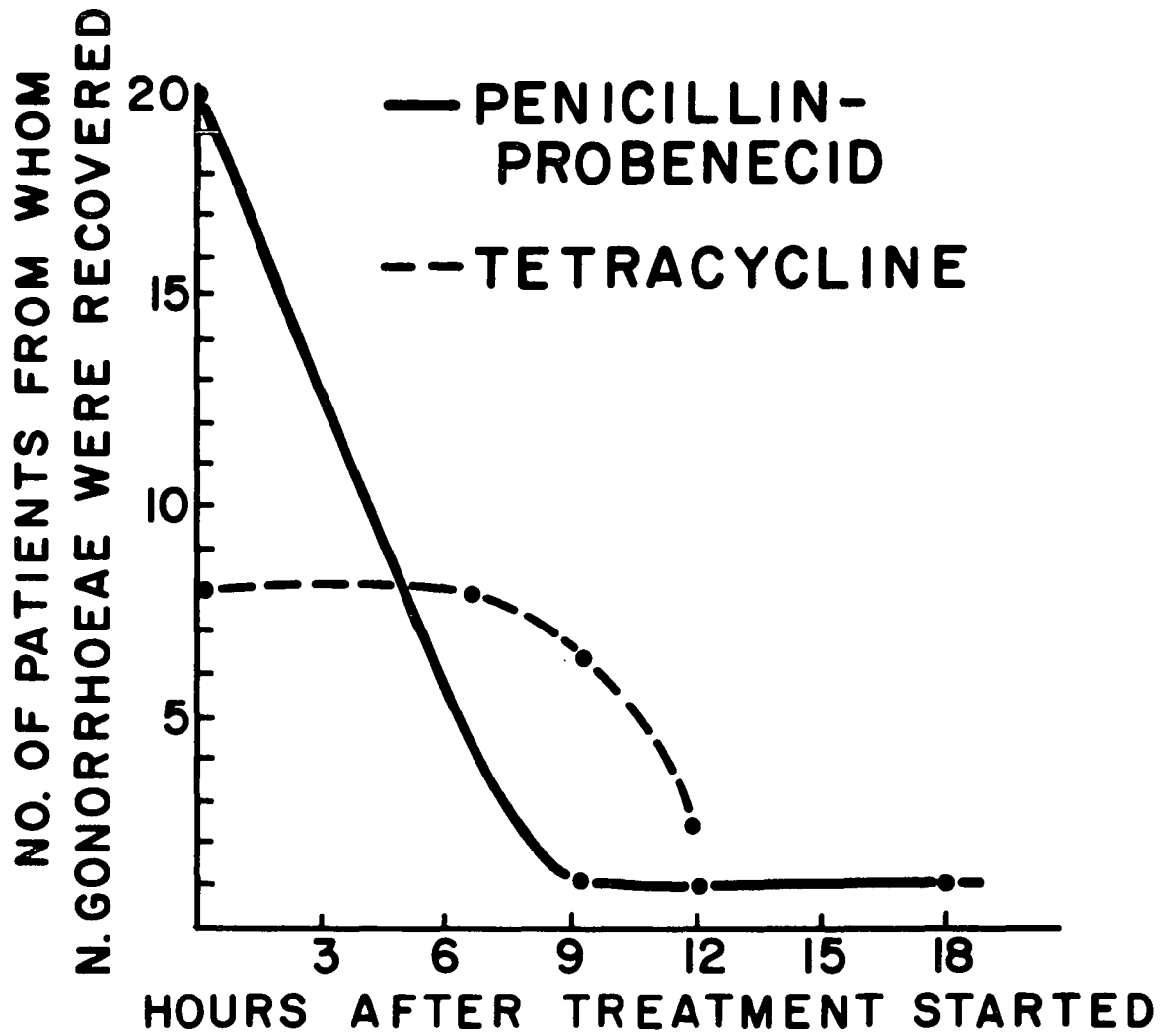
Table 2. Penicillin resistance of 115 isolates of N. gonorrhoeae as determined by growth on solid medium containing different concentrations of penicillin

Units Penicillin G. per ml of Media	Growth of <u>N. gonorrhoeae</u>			
	First Study (41 Strains Tested)		Second Study (74 Strains Tested)	
	Number	Percent	Number	Percent
0.00 (control)	41	100	74	100
0.060	26	63	57	77
0.125	17	41	46	62
0.250	12	29	24	32
0.500	11	27	8	11
1.000	1	2	2	3
5.000	0	0	0	0

Studies of Venereal Disease I. Probenecid-Penicillin Combination and Tetracycline in the Treatment of "Penicillin-Resistant" Gonorrhoea in the Male.

LT King K. Holmes, MC, USNR

Legend for Figure 1.--Duration of viability of N. gonorrhoeae after initiation of treatment with penicillin-probenecid and with tetracycline, as determined by urethral cultures.



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CHAPTER II  
STUDIES OF VENEREAL DISEASE II.  
OBSERVATIONS ON THE INCIDENCE, ETIOLOGY, AND TREATMENT  
OF THE POSTGONOCOCCAL URETHRITIS (PGU) SYNDROME.

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## Synopsis - Abstract

Post gonococcal urethritis (PGU) occurred in nearly two-thirds of men with gonorrhoea acquired in the Far East who were "successfully" treated with 2.4 million units of procaine penicillin G plus probenecid. PGU occurred less often after treatment of gonorrhoea with tetracycline, and PGU itself responded to tetracycline, suggesting a microbial etiology for the condition. The syndrome was associated to a highly significant degree with mycoplasma infection of the urethra, and occurred more often after infection with N. gonorrhoeae of lessened penicillin sensitivity. The high incidence of the syndrome, probably the second most common infectious disease among military personnel in the Far East, requires a reexamination of the traditional use of penicillin in the treatment of gonorrhoea in the male.

Key words: Post gonococcal Urethritis (PGU)

Mycoplasma hominis

T-strain mycoplasmas

L-form of N. gonorrhoeae

It is still possible to manage the treatment of "penicillin-resistant" gonorrhoea in men by using high doses of penicillin with probenecid as an adjunct when necessary. Although such treatment is "successful" in that viable N. gonorrhoeae can no longer be recovered from the urethra after treatment, the persistence of residual post gonococcal urethritis (PGU) appears to be far more common than was previously suspected. For example, in a previously reported investigation<sup>1</sup>, 63 men aboard an aircraft carrier were treated for acute gonorrhoea with a single dose of 2.4 million units of procaine penicillin G. Eighteen treatment failures resulted, but N. gonorrhoeae was eventually eradicated from all 18 with larger doses of penicillin. Two months after the treatment study one of us returned to the ship and reviewed the health records of all the patients who remained aboard. Thirteen patients had been transferred from the ship, and three had no further entries in their health records after termination of the study. Of the remaining 47 patients who had received some further follow-up, only one had been found to have recurrence or reinfection with gonorrhoea. However, 18 (38%) had developed nongonococcal urethritis (NGU) and had been treated with tetracycline by the ship's medical department.

The present study was undertaken in order to gain more information about the subsequent course of "successfully" treated gonorrhoea in a group of men who were kept from sexual reexposure to infection while at sea aboard a second large aircraft carrier. It became apparent early in the study that the incidence of PGU characterized by urethral exudate or by pyuria increases with increasing length of follow-up and probably

can be detected in the majority of cases of gonorrhoea treated with penicillin.

It has not previously been clear whether PGU represents persisting gonorrhoea, coincidentally acquired nongonococcal urethritis (NGU), secondary infection by the normal urethral flora, or a normal, slow resolution of urethral inflammation following adequate treatment of gonorrhoea. After initial penicillin therapy, it is common practice in the U. S. Armed Forces to treat PGU with a second course of antibiotics, although there is no evidence that this syndrome is caused by a microbial agent. The military "medical quarantine" which is imposed upon men with acute gonorrhoea is often prolonged in those who manifest PGU, despite lack of evidence for the transmissibility of PGU by sexual contact.

In the course of the present shipboard investigation the penicillin sensitivities of primary N. gonorrhoeae isolates were determined in looking for a relationship between the level of penicillin resistance and the development of PGU. Extensive bacteriological studies were performed in looking for a possible relationship of the urethral microbial flora to the persistence of urethral inflammation. The effect of a second course of antibiotic (tetracycline) was analyzed in several men who had residual PGU after initial penicillin-probenecid treatment. Finally, the incidence of PGU was determined over a six-month's period in a separate large group of men who also were treated for gonorrhoea with penicillin-probenecid, but who were stationed ashore and therefore may have had sexual reexposure.

## Materials and Methods

Study Population.--Three different groups of men were studied.

(1) The shipboard study group consisted of 88 men with acute gonorrhoea who were part of a crew of 5,500 men on an aircraft carrier. The diagnostic studies, treatment, and the follow-up of these men were all carried out while this ship was at sea for 30 consecutive days. Thus, there was no possibility of heterosexual reinfection.

(2) Eighty-eight shipmates of the infected men were selected from the daily sick-call line as controls for the purposes of mycoplasma and virus isolations. Thirteen of these men were rejected because of symptoms related to the genito-urinary tract. Of the remaining 75 controls, 39 (52%) admitted to sexual contact during the previous period in port.

(3) During the subsequent study, conducted from November 1966 to April 1967, 669 men with acute gonorrhoea were treated at the U. S. Naval Station Dispensary, Subic Bay, R. P., with 2.4 million units of procaine penicillin G plus oral probenecid. Of these men, the 242 who returned for reexamination after 20 days comprise the second study group.

Initial Evaluations.--The following laboratory studies were done:

(1) As previously described<sup>1</sup>, the diagnosis of gonorrhoea was established during the shipboard study by Gram-stained smears, culture, and oxidase and fermentation reactions. Human blood agar and MacConkey agar plates were inoculated with loopsful of urethral exudate in an attempt to isolate staphylococci and organisms of the so-called Tribe "Mimeae".

(2) For the isolation of mycoplasma, specimens were obtained by gently scraping the bacteriologist's loop repeatedly against the wall of the distal urethra. These scrapings were inoculated on a modified Chanock's medium<sup>2</sup>, which contained Agamma Horse Serum (Hyland Laboratories) and was adjusted to pH 6.5. These plates were incubated at 37° C. under 95% N<sub>2</sub>-5% CO<sub>2</sub> gas, and were examined after five and ten days for growth. Isolates were transferred to broth of the same composition as the modified agar medium except agar was omitted, and were frozen in liquid nitrogen to hold for subsequent identification. The agar surface of each isolation plate was then stained with Diene's stain and examined under 200x magnification to aid in the recognition of very small colonies. Specimens from all patients with gonorrhoea in the shipboard study were again cultured for urethral mycoplasmas five days after treatment.

Mycoplasma-isolates were identified by growth-inhibition with homologous antiserum obtained from Robbin Laboratories, Inc., and identifications were confirmed with rabbit antisera prepared at Naval Medical Research Unit No. 4.

(3) Specimens of urethral exudate or scrapings obtained from shipboard patients and controls were inoculated into Hank's balanced salt solution with 15% skimmed milk and then were maintained frozen for future viral studies.

(4) During the dispensary study, the diagnosis of gonorrhoea was established by Gram-stained smears of urethral exudate.

(5) In both the shipboard and the dispensary groups, the possibility of persisting N. gonorrhoeae infection after 20 days was



excluded by culture examination of all patients with PGU characterized by urethral exudate.

Treatment.--Shipboard patients were treated either with oral tetracycline or with procaine penicillin G and probenecid<sup>1</sup>. At the Naval Station Dispensary all patients were treated with the procaine penicillin G and probenecid regimen.

Follow-up Evaluation.--All 88 patients in the first study were reexamined at five-day intervals for a minimum of 20 and a maximum of 30 days following treatment. Only two of over 400 scheduled reexaminations were missed. At the first five-day follow-up, a second specimen was obtained for mycoplasma-isolation. During each follow-up examination, after an attempt to demonstrate urethral exudate, a two-glass urinalysis was performed as follows: The first ten ml of the morning specimen were collected in a graduated centrifuge tube; the remainder of the urine was collected in a urine bottle and a ten ml sample of this was placed in a second tube. Each tube was centrifuged, the supernatant was decanted, and the remaining 0.5 ml of sediment was examined microscopically. The number of white blood cells in three successive high dry fields (WBC/HDF) were recorded. The two centrifuge tubes were coded so that the technician did not know which contained the first ten ml of urine, although the first specimen was obviously more turbid in most cases, even in the absence of pyuria. Urines from fifteen normal controls were similarly examined to establish normal baseline values for this method. Since none of these control urines contained over ten WBC/HDF, it was arbitrarily decided that 20 or more WBC in any one of three HDF was significantly above normal. Thus, 20 or more WBC in a HDF

is considered to represent pyuria in this study.

Men treated with penicillin and probenecid in the Naval Station Dispensary were followed in the same way, except that they were examined only one time, twenty days after treatment. Specimens for mycoplasma and viral studies were not obtained from this group.

During the follow-up of patients aboard the aircraft carrier, the examining physician and laboratory technicians did not know which treatment each patient had received initially, in order to avoid bias in the subsequent examinations and comparison of the incidence of PGU in each group.

Treatment of PGU with Tetracycline.--Shipboard patients treated with penicillin and probenecid who had either urethral discharge or first-glass pyuria of 20 or more WBC/HDF, when reexamined 20 days after initial treatment, were considered to have PGU. These men were studied to determine the effect of tetracycline on PGU. In the order in which they completed their 20 days of follow-up, these patients were alternately assigned either to treatment with tetracycline, 500 mg orally every six hours for 16 doses, or to no treatment at all. Both groups were then reevaluated after an interval of five to seven days.

### Results

Incidence of PGU.--PGU was present 20 days after therapy in nearly two-thirds of 58 men treated aboard ship with penicillin and probenecid, but only in about one-fourth of the 30 men treated with tetracycline (Fig. 1). However, of the total of 45 men from this shipboard group with objective evidence of PGU, only 14 were actually symptomatic. Of the 31 men who had demonstrable exudate, 15, or about one-half, had only

small expressible drops or film of whitish or clear fluid. These minimal discharges were considered significant since they were always associated with pyuria of 20 or more WBC/HDF. Figure 2 shows the progressive development of urethritis in 37 patients who had PGU 20 days after treatment with penicillin-probenecid. It can be seen that many patients pass through a period of several days after eradication of the gonococcus, during which they have no objective evidence of urethritis. Only after two or three weeks does PGU appear in these men. It is possible that a longer period of follow-up might have disclosed other delayed cases but a longer follow-up was not possible because of the ship's schedule.

The findings in the dispensary group, although based on less extensive follow-up studies, substantiate the high incidence of PGU in men treated for gonorrhoea with penicillin and probenecid. In 242 cases who were reexamined 20 days after treatment, 124 (51%) had residual post-gonococcal urethritis. It was possible to locate eleven patients and reexamine them at intervals varying from 31 to 60 days after treatment. Eight of these eleven had a discharge or pyuria.

Relationship Between PGU and Gonococcal Penicillin Sensitivity.-- It is conventional at the present time to define "lessened penicillin sensitivity" as the ability of N. gonorrhoeae to grow in the presence of 0.06 units of penicillin G per ml of medium, since resistance to this concentration was almost unknown before 1954. Penicillin sensitivities were determined on strains of N. gonorrhoeae isolated from 49 of the 58 shipboard patients treated with penicillin and probenecid. As previously reported,<sup>1</sup> 40 of the 49 strains showed lessened sensitivity to penicillin. With regard to the incidence of PGU among these 49 patients, it is

noteworthy that none of the patients with penicillin-sensitive organisms had a discharge after 20 days' follow-up, and only one had pyuria (Table 1). In contrast, 20 (50%) of the 40 patients with less sensitive organisms had a discharge during follow-up, and 30 (75%) had pyuria.

Relationship Between PGU and Urethral Mycoplasma.--As is shown in Table 2, urethral mycoplasmas were recovered from 17 of 75 controls (23%) and from 25 of 88 patients with gonorrhoea (28%). However, when the repeat-cultures, obtained after 5 days from patients only, are excluded, the isolation rates were even more comparable: 23% from controls and 22% from patients. The isolation-rate was higher from the controls who admitted to sexual intercourse during the previous liberty period (12/40 or 30%) than from controls who denied sexual intercourse in the previous liberty period (5/35 or 14%).

Most of the isolates were classical large-colony mycoplasmas. Thirteen of the 17 isolates from controls and 16 of the 25 isolates from patients were received in viable condition after transportation from the ship to Honolulu. All 29 were identified as M. hominis by growth-inhibition with homologous antiserum. Several isolates from patients and controls formed small colonies which were only 10 to 20 microns in diameter. Mycoplasma which form colonies of this size have been termed "T-strains".<sup>4</sup> Usually these were seen as satellites adjacent to the large colonies of M. hominis, but a few small colony isolates were not associated with classical large colonies. It was not possible to maintain any small colonies through the first subculture in broth or on solid medium. Some small colonies appeared to revert upon subculturing to larger colonies which were identified as M. hominis. However, this

might have been due to inadvertent contamination, since the small colonies were very close to large colonies on the primary isolation plate.

It was interesting to note that, from patients treated with tetracycline, mycoplasmas were isolated only from pretreatment specimens whereas they were recovered from both pre- and post-treatment specimens from patients in the penicillin-probenecid group (Table 3).

A possible association between the presence of urethral mycoplasmas and subsequent development of PGU is presented in Table 4. The association is statistically significant ( $P < .05$ ) whether classical mycoplasmas are considered alone or all mycoplasma isolates (classical and T-strain) are considered together in relation to PGU. For reasons to be discussed, the relationship between all mycoplasmas and PGU has been presented in Table 4. Of the 17 patients in the penicillin-probenecid group who harbored either classical or T-strain mycoplasmas or both, on their first or second urethral culture, 16 (94%) developed PGU within 20 days. Among the remaining 41 patients from whom mycoplasmas were not recovered, only 21 (51%) were subsequently found to have PGU.

Treatment of PGU with Tetracycline.--Twenty-nine shipboard patients who had been treated with penicillin-probenecid, and who had either urethral discharge or first-glass pyuria of 20 or more WBC/HDF after 20 days' follow-up, were studied to compare the course of PGU treated with tetracycline with the course of untreated PGU. The treated group (15 patients) and the untreated group (14 patients) were reevaluated after 5 to 7 days. PGU was considered to have been "suppressed" if all evidence of demonstrable discharge and symptoms had disappeared and the

number of WBC in the first-glass urinalysis had decreased to no more than 10 per HDF. According to these criteria, only one of the 14 patients who received no treatment showed spontaneous suppression of his PGU, whereas PGU was suppressed in 14 of the 15 patients given tetracycline. A longer follow-up was not possible because the ship then reentered port. Therefore, it is not certain whether the suppression of PGU observed after the 4-day tetracycline regimen was temporary or actually represented a permanent cure.

#### Discussion

PGU occurs within 20 days in nearly two-thirds of men treated for gonococcal urethritis with the penicillin-probenecid regimen used in the shipboard study. This regimen is nearly 100% curative, in terms of eradication of N. gonorrhoeae.<sup>1</sup> The incidence in the dispensary group may have been lower than in the shipboard group because first morning urine specimens were more difficult to obtain from the former group. Of the 672 gonorrhea patients treated in the dispensary group, the incidence of PGU was determined only for the 242 patients who actually returned for the 20 day follow-up. It might be argued that those who returned did so because of the presence of PGU. However, this is not thought to have created a significant bias, since most of those who did not return had been transients and had already left the area; and since only about one-third of the cases of PGU appear to become symptomatic within the 20-day period of follow-up.

Etiology of PGU.--The evidence presented above relating PGU to lessened penicillin sensitivity of the infecting strain of N. gonorrhoeae, and to mycoplasma infection of the urethra, is indirect and suggestive

rather than conclusive. Nonetheless, these relationships appear to warrant further discussion.

The possibility that variant forms of N. gonorrhoeae may produce PGU cannot be overlooked. The unexpected association between this syndrome and the lessened penicillin susceptibility of the infecting strains of gonococci could be related to the induction of gonococcal L-forms by penicillin. The concentration of penicillin has been found to be quite critical for the in vitro-induction by penicillin of L-forms of other microorganisms. For example, at low concentrations of penicillin pneumococci survive in the typical bacterial form, but when excessively high concentrations are used neither L-forms nor typical bacterial forms survive.<sup>5</sup> L-form induction occurs only within a relatively narrow range of penicillin concentrations. Similarly, the concentrations of penicillin which inhibit growth of L-forms of several strains of streptococci and staphylococci range from 2 to 1000 times the concentrations required for inhibition of bacterial growth.<sup>6</sup> The lethal concentrations of penicillin for L-forms of 4 strains of staphylococci were only two to eight times the concentrations which were lethal for bacterial growth. Only recently, Roberts<sup>7</sup> reported the successful production of stable L-forms of N. gonorrhoeae. The minimal inhibitory concentrations of various penicillins were  $10^3$  to  $10^5$  higher for the L-form than for the bacterial form of one strain. The very fact that L-forms tend to appear only within a certain limited zone on penicillin gradient plates<sup>8</sup> suggests that the concentration of penicillin required for L-form production is limited to a certain range for any given strain of N. gonorrhoeae.

Thus it is conceivable that the blood and tissue concentrations of penicillin achieved with the doses of penicillin-probenecid used in these studies were too high to induce stable L-forms of penicillin-sensitive gonococci, but were not too high for L-form induction of those gonococcal strains with lessened sensitivity. In this context, it is interesting that Ashamalla et al.<sup>9</sup> found a higher incidence of residual asymptomatic urethritis after treatment with 2.4 million units of benzathine penicillin G and PAM (16.2% PGU) than after treatment with 2.4 million units of procaine penicillin G (11.8%). Although the difference was not statistically significant it is compatible with the hypothesis that the latter regimen, by producing higher blood-levels of penicillin, was less suitable for the induction of stable L-forms of N. gonorrhoeae. It is surprising that the PGU syndrome, apparently of common occurrence now, has not attracted more attention during the years since the advent of penicillin therapy for gonorrhoea. One possible explanation is that it did not occur as often when gonococci were universally sensitive to low concentrations of penicillin. However, this conclusion is merely speculative. L-forms have not yet been convincingly demonstrated by acceptable methods in patients with PGU.

The higher incidence of PGU after initial penicillin therapy, as compared with initial tetracycline therapy, is compatible with the L-form induction hypothesis, since tetracycline does not induce L-forms of other microorganisms in vitro. This difference in incidence of PGU after the two treatments might also be due to one or more microbial agents other than N. gonorrhoeae which are susceptible to tetracycline but not to the single-dose penicillin regimen used in this study. The prompt suppression



of all objective manifestations of PGU in 14 of 15 patients treated with tetracycline also strongly suggests that this syndrome is due to the presence of a microbial agent which is susceptible to tetracycline, rather than due to "healing" with slowly-resolving, noninfectious inflammation.

It is tempting at this point to suggest that the association of urethral mycoplasmas with PGU is a casual one. As shown in Table 3, urethral mycoplasmas were eradicated by tetracycline, but not by penicillin. The frequently delayed onset and indolent course of PGU are comparable to the course of infection characteristic of numerous other pathogenic strains of mycoplasma isolated from animals and humans.<sup>10,11</sup>

In previous studies reviewed by Hayflick and Chanock,<sup>12</sup> sometimes urethral mycoplasmas have been found more often in patients with non-gonococcal urethritis than in normal controls. These results could have been due to significant dissimilarities between patients and controls, or to an alteration of the urethral flora in the different patient groups by an unrecognized pathogenic agent which produced conditions more favorable to mycoplasmas. In our shipboard study, the prevalence of urethral mycoplasmas was comparable in the controls and the gonorrhea patients. Urethral mycoplasmas were recovered more often from controls who admitted to recent sexual intercourse than from those who denied exposure. Although this difference was not statistically significant, other workers have noted a correlation between the presence of urethral mycoplasma and sexual activity.<sup>13</sup> In the penicillin-probenecid treatment group under discussion, the 41 patients without urethral mycoplasmas served essentially as additional "internal controls" with respect to the

late development of PGU. Thus, the association shown in Table 4 between the initial presence of mycoplasmas and the subsequent presence of PGU is of particular significance.

Although 13 of the controls had complaints referable to the urogenital tract, none had objective evidence of anterior urethritis. If mycoplasmas contribute to the etiology of PGU, it is difficult to understand why urethritis was not produced in the controls. It may be that a damaged urethra, recovering from a gonococcal infection, provides the unique environment necessary for pathogenic expression of mycoplasma infection. Microbial synergism does seem to occur in certain animal mycoplasma infections,<sup>14,15</sup> and, under different kinds of stress, latent mycoplasma infections may be "lighted up" in the experimental animal.<sup>16,17</sup> Thus, in the sexually active male, the urethral mycoplasmas might be regarded as a part of the normal urethral flora but can become secondary pathogens in the damaged urethra.

No distinction was made between classical M. hominis and "T-strain" mycoplasmas with respect to the possible role of these organisms in causing PGU. Shepard<sup>18</sup> and Ford<sup>19</sup> have presented evidence which suggests that T-strain mycoplasmas play a unique role in the causation of non-gonococcal urethritis. However, the T-strains are difficult to work with and have not yet been adequately characterized as a single species of mycoplasma. In fact, a recent report indicates that the T-strains are an antigenically heterogeneous group.<sup>20</sup> During the present study, these small colonies were generally found only in association with classical colonies of M. hominis, and then usually as rare satellite colonies adjacent to the larger colonies. It may be that in some cases the small

colonies arose from strains of M. hominis as genetic variants which were less well adapted to the culture environment. In any event, their relationship to the "T-strain" isolated by Shepard and Ford is not clear. There is no evidence as yet that "T-strain" mycoplasmas are more often associated with PGU than are M. hominis, if they are, in fact two different species of mycoplasma.

Whether PGU is caused by a variant of N. gonorrhoeae, by mycoplasmas, or by some other still unrecognized member of the normal urethral flora, the frequency with which the syndrome now occurs may require a review of present concepts of gonorrhea treatment in the male. PGU is now very probably the second most common infectious disease seen by U. S. military physicians, second only to gonorrhea. Although the relationship of PGU to nongonococcal urethritis is not entirely clear, a substantial proportion of the cases of nongonococcal urethritis encountered in military personnel unquestionably are actually PGU. A discussion of this relationship will be presented in a subsequent publication.

Although penicillin is still generally considered to be the drug of choice in the treatment of gonorrhea, the occurrence of PGU in two-thirds of patients treated with penicillin must raise serious questions about the wisdom of this concept. It is hoped that our findings will serve as a stimulus to the evaluation of other antibiotics in the treatment of gonorrhea. It is essential to distinguish between treatment failure, meaning persistence of demonstrable N. gonorrhoeae, and PGU in discussing the treatment of gonorrhea in the male. The principal advantage of penicillin, aside from the vast clinical experience with the drug, is its effectiveness in a single dosage in eradicating N. gonorrhoeae. The

single-dose treatment is important from a public health standpoint, but optimal treatment must also minimize PGU if a preventive approach to the problem of nongonococcal urethritis is to be attained.

If PGU is in fact related to gonococcal L-forms, or to mycoplasma infection, then the antibiotics most likely to be effective in preventing PGU would be those which do not act by inhibiting formation of cell walls. Tetracycline now appears to be the most effective drug for the treatment of gonorrhoea in the male, in terms of prevention of PGU. However, since a single-dose regimen of tetracycline has not yet been shown to be adequate either in eradication of N. gonorrhoeae or in prevention of PGU, tetracycline should be given as a multiple-dose regimen and hence can be given only to those patients who can be closely supervised or can be relied upon to administer their own medication. At the present time an acceptable approach to most patients would include initial treatment with a single dose of penicillin with probenecid, if necessary; follow-up examination within one week to detect treatment failures; and subsequent treatment with tetracycline of patients who return with symptomatic PGU. The dose of tetracycline to be used in such patients has not been established, but unpublished data<sup>20</sup> suggest that the administration of 1500 mg initially, followed by 500 mg every six hours for seven days, is more successful than the four-day regimen used in our shipboard study.

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Generic and Trade Names of Drugs

Probenecid - Benemid

Table 1. Relationship between PGU and penicillin sensitivity of N. gonorrhoeae recovered from 49 shipboard patients treated with penicillin-probenecid\*

Gonococcal Penicillin Sensitivity	Total Number Patients	20 Days Post-treatment No. with Discharge	No. with Pyuria or Discharge
Sensitive to 0.06 units/ml	9	0	1
Resistant to 0.06 units/ml	40	20	30
Significance		p<.02	p<.02

\*Penicillin sensitivities were determined for 49 of the 58 isolates from patients treated with penicillin-probenecid.

Table 2. Number of individuals harboring classical and "T-strain" colonies of mycoplasma among 88 men with gonorrhoea and 75 controls

	Gonorrhoea* (%)	Controls (%)
Classical colonies only	12 (13.6)	9 (12.0)
"T-strain" colonies only	3 ( 3.4)	1 ( 1.3)
Both classical and "T-strain"	10** (11.4)	7 ( 9.3)
Totals	25 (28.4)	17 (22.7)

\*Includes initial and repeat cultures. Initial cultures of gonorrhoea patients yielded only 19 isolates (22%), which was comparable to controls (23%) .

\*\*In 8 cases, large and T-strain colonies were present simultaneously on same primary isolation plate. In 2 cases, large colonies were present only on initial plate and "T-strain" only on repeat plate, or vice versa.



Table 3. Isolation of mycoplasmas from 58 gonorrhoea patients given penicillin-probenecid and from 30 patients given tetracycline

Time of Isolation	No. Individuals Yielding Mycoplasmas	
	Penicillin-Probenecid	Tetracycline
Pretreatment only	5	8
Both Pre- and Post-treatment	6	0
Post-treatment only	6	0
Totals	17	8

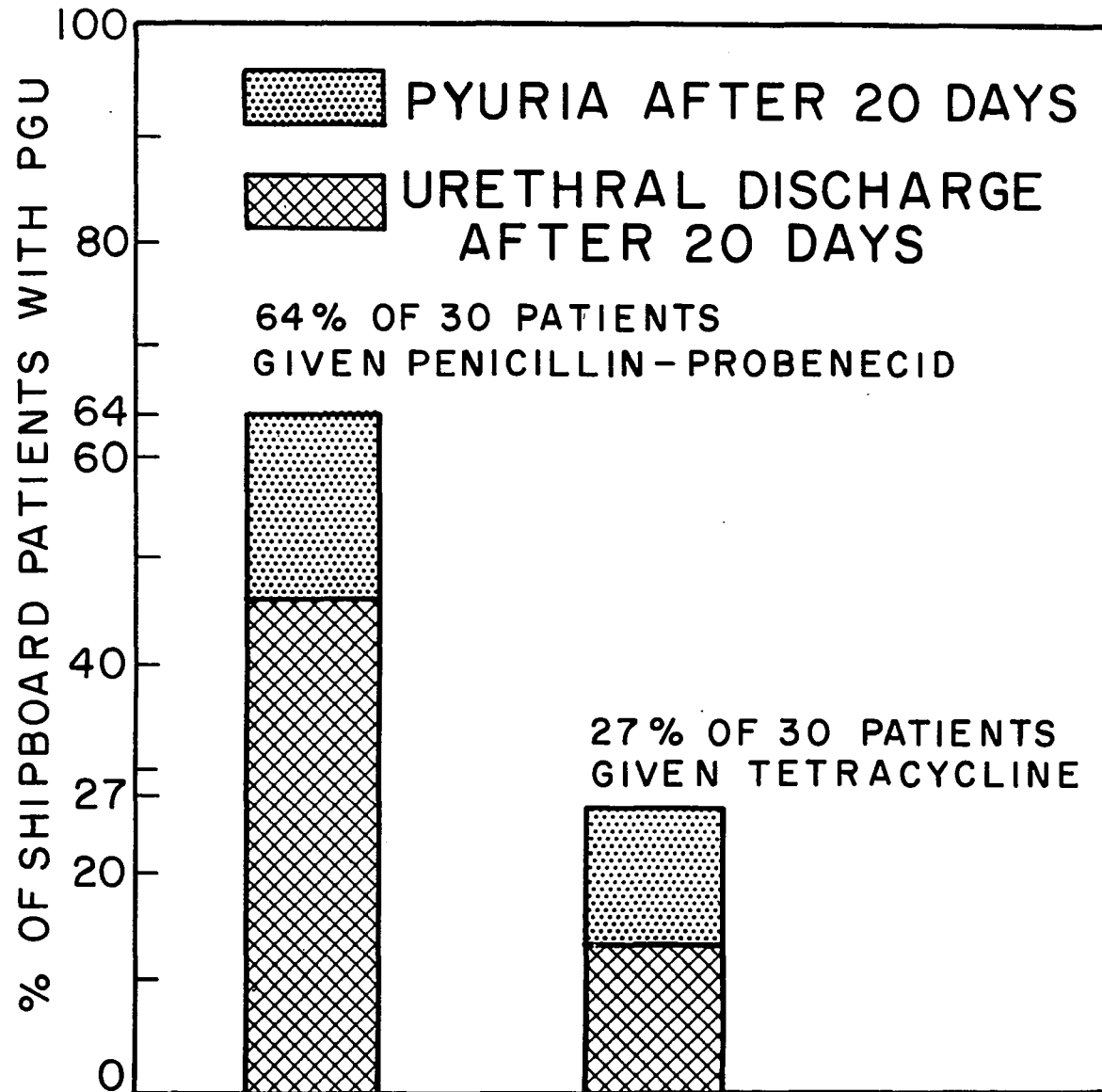
Table 4. Relationship between the presence of mycoplasmas and PGU in 58 patients treated with penicillin-probenecid

Presence of Mycoplasma	Total Number	Patients	
		Number	Percent
Present	17	16	94
Absent	41	21	51
Significance		$p < .05$	

Studies of Venereal Disease II. Observations on the Incidence,  
Etiology, and Treatment of the Postgonococcal Urethritis (PGU) Syndrome.

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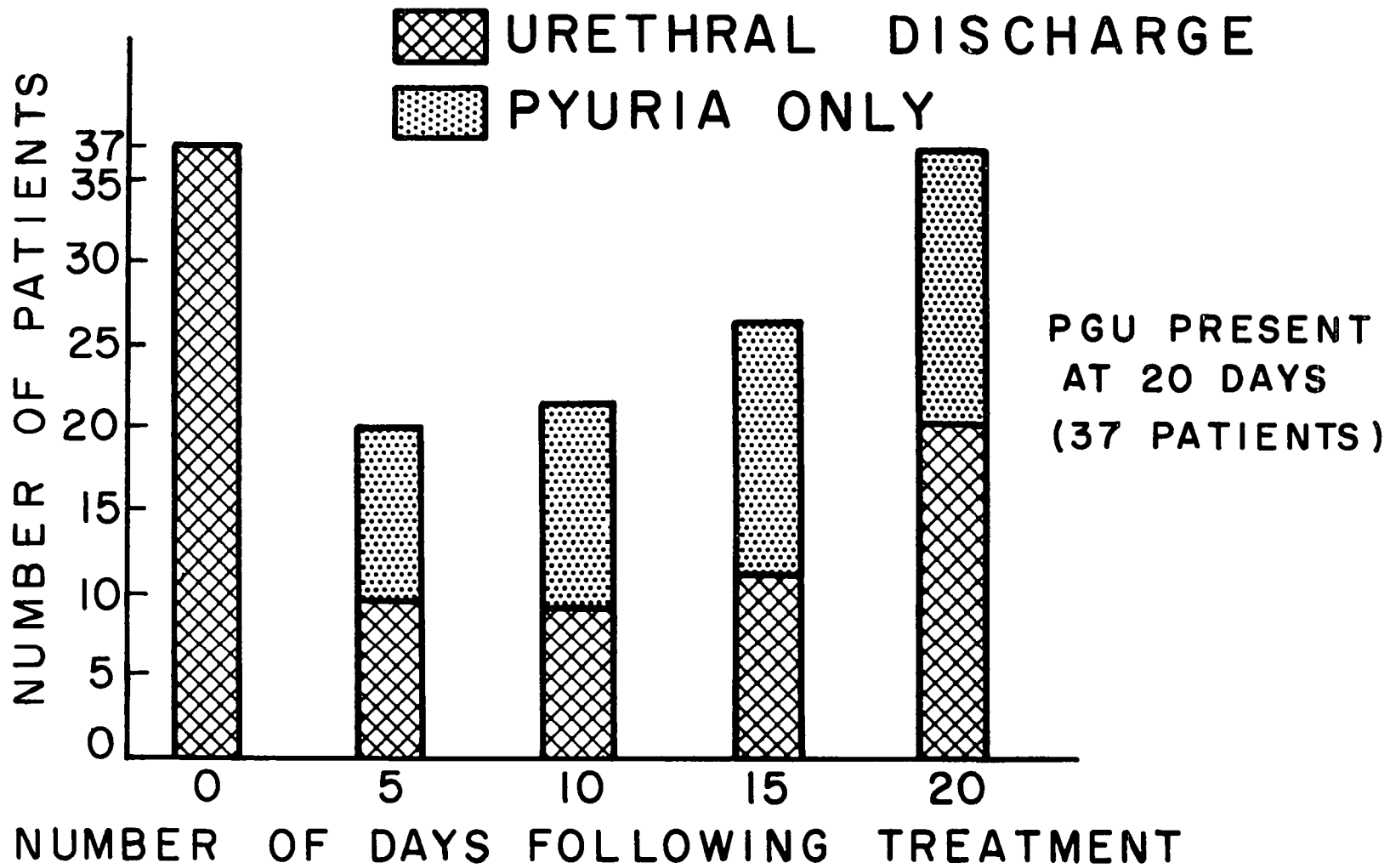
Legend for Figure 1.--Incidence of postgonococcal urethritis  
(urethral discharge with pyuria alone) in each treatment group.



Studies of Venereal Disease III. Observations on the Incidence,  
Etiology, and Treatment of the Postgonococcal Urethritis (PGU) Syndrome.

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Legend for Figure 2.--Rate of development of either urethral discharge with pyuria or pyuria alone in 37 patients who manifested PGU 20 days after treatment with penicillin-probenecid.



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CHAPTER III  
AN ESTIMATE OF THE RISK OF MEN  
ACQUIRING GONORRHEA BY SEXUAL CONTACT WITH CHRONICALLY  
INFECTED FEMALES

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It has been well known since World War II that not all men who have sexual intercourse with a woman who is infected with N. gonorrhoeae actually acquire gonorrhea.<sup>1</sup> A quantitative estimate of the risk of transmission associated with sexual contact is of interest in achieving a basic understanding of the factors which are involved in transmissibility of gonorrhea. This information would be essential as a baseline for comparison in the evaluation of antibiotic or mechanical prophylactic measures. From the public health standpoint, such an estimate would also be useful. For example, in the control of syphilis, the demonstration of transmission in 9.2% of contacts involving an individual with syphilis<sup>2</sup> has been partly responsible for the adoption of the highly successful techniques of epidemiologic treatment and of cluster contact tracing. Comparable information regarding the transmissibility of gonorrhea has not been readily available in the past, mainly because of the difficulty involved in establishing a diagnosis of gonorrhea in female contacts. The study of urethritis in U. S. Navy personnel aboard an aircraft carrier was performed in conjunction with a study of gonorrhea infection in Filipino prostitutes who were consorts of the men during their liberty periods in Subic Bay, R. P. Data obtained during these two studies have permitted an estimate of the risk of transmission of gonorrhea from female to male during sexual intercourse.

#### Methods

Study Population.--The Navy men were enlisted members of a 5500 man crew aboard a large aircraft carrier.<sup>3</sup> During their liberty periods in Subic Bay, their female consorts were the 4800 licensed Filipino

"hostesses" in Olongapo, a town with a population of about 100,000 which borders the Subic Bay complex. Because of the increasing numbers of gonorrhoea cases arising from contact with Olongapo hostesses, the Navy was invited by Olongapo health officials to send an investigating team to assist in controlling the problem. The hostesses were required to report at regular intervals to a city examination center where specimens were obtained for cultures for N. gonorrhoeae. More than 1300 of these hostesses were randomly selected for inclusion in a group which was examined by members of the Navy's investigating team.

Diagnostic Methods.--In the female group initial studies, which will be reported later, were carried out to determine the most efficient diagnostic method for detecting the presence of chronic gonorrhoea infection in these hostesses. It was found that endocervical scrapings, obtained with a stiff wire loop and inoculated onto Thayer-Martin medium,<sup>4</sup> gave the highest yield of positive cultures for N. gonorrhoeae. Use of this medium resulted in a very low incidence of false negative results with essentially no false positive results.

Using this method, the 1300 hostesses were examined an average of three times each over a period of six weeks, in order to establish the prevalence of gonorrhoea in the overall hostess population.

In the male group studies were carried out aboard the carrier when it put out to sea after a six day liberty period in Subic Bay. The incidence of gonorrhoea which resulted in these men, from the six day period of exposure, was determined performing diagnostic studies, as previously described,<sup>3</sup> on all men with symptoms of urethritis who reported for medical treatment during the liberty period and during the following 30 days at sea.

In order to determine the number of sexual exposures among the crew during the six day liberty period, it was possible to ask most of the crew members to fill in a confidential questionnaire in conjunction with a tuberculin skin testing program which involved the whole crew. The men were asked whether they had sexual contact during the last liberty period, and if so, with how many different women, and how many "times" altogether. A smaller group of 131 men who admitted sexual contact were asked if they had used mechanical prophylaxis (Condom). All patients with gonorrhoea or NGU and 88 randomly selected controls were also asked if they had attempted self-treatment or self-prophylaxis with antibiotics.

From the above data a formula for the risk of acquiring gonorrhoea by sexual contact with an infected woman was derived:

1. Number of expected male cases = Number of crew members X Proportion of crew admitting sexual contact X Proportion not using Condom X mean numbers of different consorts of men admitting sexual contact X Proportion of women infected.

$$2. \text{ Risk} = \frac{\# \text{ observed cases}}{\# \text{ expected cases}}$$

### Results

Prevalence of Gonorrhoea in Females.--The prevalence of gonorrhoea infection in the hostess population examined by the Navy team is defined as the percent of endocervical cultures which yielded N. gonorrhoeae on any given day. The daily prevalence was nearly constant. The mean prevalence, based on the combined figures over several weeks time, was 11.1%. This figure was consistent with the prevalence determined simultaneously by the city health officials, who obtained

thousands of specimens under the direct supervision of the Navy team from the remainder of the 4800 hostesses who were not included in the study group.

Incidence of Gonorrhoea in Men.--A total of 88 cases of acute gonorrhoea were seen during and following the liberty period. All of these cases occurred in enlisted men, so only the results of questionnaires and interviews of enlisted men are considered in the following computations.

Results of Questionnaires and Interviews.--Of a total of 4913 enlisted men on board at the time of the study, questionnaires were completed by 4605. The number of enlisted men admitting to sexual contact with a prostitute at any time during the preceding seven months of the cruise was 3277 (71.2%). The number admitting contact during the six day liberty period was 2052 (43.6%). A mean of 1.2 different consorts were claimed by men admitting contact during the six day liberty. The median number of "times" the men had intercourse was three; this means  $3 \div 1.2$  or approximately 2.5 "times" per consort. Of the 131 men interviewed regarding mechanical prophylaxis, 23% claimed that they had used a condom. Self treatment or self-prophylaxis was denied by all 88 patients with gonorrhoea, by all 88 controls, and by 103 of 104 patients with NGU.

Thus, according to formula (1):

Number of expected cases =  $4913 \times .436 \times .77 \times 1.2 \times .11 = 218$ .

The risk of infection of a male who has sexual intercourse 2.5 "times" with an infected female is given by formula (2):

Risk =  $88 \div 218 = 40.3\%$ .

## Discussion

The above exercise is limited by our inability to control a number of variables. The most important of these is the possibility of self-treatment which could falsely lower the calculated risk by lowering the number of observed cases in men. Antibiotics were readily available for purchase in Olongapo. However, the social stigma associated with gonorrhoea was minimal in these men, and the only punitive measure permitted was confinement to the ship during the remainder of the liberty period. Since the onset of symptoms in most men occurred at the end of the liberty period or after the ship put out to sea, most individuals would have had little motivation to attempt self-treatment. Patients and controls denied self-treatment but the possibility exists that they lied. The critical test would be the screening of sera from a representative group of men for bacterial-inhibiting properties, due to the presence of antibiotics in the blood. Sera were obtained from most patients and controls for this purpose, and will be screened in the near future by the Sarcina lutea cup-plate method as outlined by Grove and Randall.<sup>5</sup>

A second variable is the manner in which the men and women paired off. The calculations were based on the assumption that this was a random process. However, it may be that the most effective hustlers were also the most ineffective hustlers; that is, those women who were most promiscuous might be expected to have a higher prevalence of gonorrhoea. Hostesses who were employed in semi-brothels had a consistently higher prevalence of gonorrhoea than hostesses employed in establishments which did not permit prostitution within the premises.

This bias would have tended to falsely increase the calculated risk. However, it is doubtful that this bias would have greatly affected the computation, since even in the semi-brothels, the prevalence of gonorrhoea did not exceed 16%.

A third variable is the honesty with which the enlisted crew members filled out the questionnaire. The results are thought to be valid, since they agreed closely with the results of personal interviews of the 88 controls, and with the results of a similar questionnaire administered aboard a second aircraft carrier.



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## CHAPTER IV

## STUDIES OF VENEREAL DISEASE III.

## TREATMENT OF NONGONOCOCCAL URETHRITIS:

## A DOUBLE-BLIND COMPARISON OF TETRACYCLINE AND PLACEBO.

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views of the Navy Department or the Naval Service at large.

Since the discovery of N. gonorrhoeae by Neisser in 1879, the problem of nongonococcal urethritis (NGU) in males has become more and more puzzling. The etiology, epidemiology, pathogenesis, and the optimal treatment of the syndrome remain undefined. Previous investigations of the therapy of NGU<sup>1-5</sup> usually have shown an apparently beneficial effect of the tetracyclines in ending clinical symptoms and urethral exudate. However, these studies have had several shortcomings, such as (1) reliance upon the examining physician's subjective evaluation in establishing the diagnosis of NGU, and in classifying patients as "failures" or "cures" following treatment; (2) inadequate experimental controls, and failure to use a double blind design in treatment and follow-up examinations; (3) reliance upon patients to self-administer medication without supervision; (4) inability to prevent sexual reexposure and reinfection of patients after treatment; and (5) incomplete follow-up, with compilation of treatment-results based only upon the progressively diminishing proportion of patients who return for follow-up examination.

Although direct comparisons have been made between the effects of placebo and of tetracycline upon the course of NGU, <sup>3,4,5,6</sup> these studies have also suffered from the above shortcomings. Several have led even to conflicting conclusions. For example, Willcox<sup>5</sup> found that tetracycline was significantly more effective than placebo in eradicating NGU, whereas Laidig and Berg<sup>6</sup> concluded that placebo or pyridium were nearly as effective as antibiotics.

The present study was designed to eliminate many of the problems mentioned above, in the hope of obtaining valid information about the effect of tetracycline treatment upon NGU.

## Materials and Methods

Study population.--The patients were members of a 5500 man crew aboard a large aircraft carrier. This ship had been at sea for 33 consecutive days before entering Subic Bay, R. P., for six days of liberty and then returned to sea for 30 more consecutive days, eliminating the opportunity for sexual reexposure of the crew. All men with symptoms of urethritis who presented themselves at the daily sick call after the ship left Subic Bay were included in the treatment evaluation.

Initial evaluation.--As previously described,<sup>7</sup> patients from whom N. gonorrhoeae was not isolated during three successive daily examinations were placed in the NGU evaluation group. Of these men, only those with demonstrable urethral exudate were treated. When the diagnosis of gonorrhea had been excluded, men with urethral exudates were alternately assigned to treatment with tetracycline hydrochloride or with a lactose placebo. Tetracycline was administered in a 1500 mg initial oral dosage, followed by 500 mg each six hours thereafter for four days. Later in the study, in order to evaluate the importance of the duration of treatment, this schedule was changed to 1500 mg initially followed by 500 mg given each six hours for seven days. The lactose placebo was administered as six capsules initially, followed by two capsules each six hours for four or seven days, to correspond with the tetracycline regimen. Every dose of placebo and tetracycline was administered to each patient next to a water fountain by a member of the investigating team.

Men who had symptoms of urethritis without objective evidence of exudate were not treated, but were followed together with the treated patients.

Follow-up evaluation.--Patients were followed at five-day intervals after initiation of treatment. At each follow-up a first morning urine was submitted for a first-glass urinalysis using a method which had previously been standardized.<sup>8</sup> All patients were examined before the specimen was obtained by one physician who did not know which treatment the patient had received. At the first follow-up examination, patients who were not responding to treatment were switched to a new treatment according to a semi-crossover schedule. Patients not responding to placebo were re-treated with either the four day or the seven day tetracycline regimen. Patients not responding to tetracycline were re-treated with erythromycin, 500 mg each six hours for four days. The following criteria were adhered to in the physician's decision whether or not to re-treat:

1. Symptoms and exudate unchanged: re-treat
2. Symptoms unchanged in patient who had never demonstrated exudate: re-treat
3. Symptoms improved, but exudate unchanged: re-treat
4. Symptoms unchanged, but exudate improved: no re-treatment
5. Symptoms improved in patient who never manifested exudate: no re-treatment
6. Symptoms improved and exudate improved: no re-treatment

At the ten-day follow-up examination and at all subsequent examinations, any persistence or recurrence of symptoms or exudate dictated re-treatment. The presence or absence of pyuria was not known by the examiner, and did not affect the decision of whether or not to re-treat. This information was used only in the final

analysis of the effects of tetracycline and of placebo. Previous studies using the present method<sup>8</sup> have shown that first-glass pyuria of 20 or more white blood cells in any one of three high-dry fields is abnormal and is not found in normal patients who do not have urethritis.

### Results

A total of 206 men with symptoms of urethritis were evaluated after the ship left Subic Bay. The diagnoses for these men, based on examination, bacterial cultures, and first-glass urinalyses, are listed in Table 1. Only the 96 men with NGU characterized by urethral exudate were treated with placebo or tetracycline. Two of them were transferred early in the study, leaving 94 men for evaluation.

The results of the double-blind, modified cross-over comparison of tetracycline with placebo are presented in Table 2. The superiority of both tetracycline regimens over placebo is apparent, at least within the limited time-span encompassed by this study. Of the four patients who required re-treatment with erythromycin after failing to show a clinical response to the four-day tetracycline regimen, three had previously been treated for NGU within the past six months. Only one of these four patients responded to the erythromycin.

The duration of follow-up, which ranged from 5 to 25 days, depended only upon when the patients were started on treatment, since all patients were followed at five-day intervals from the onset of treatment to the end of the 30-day cruise. When the ship reentered port, the study was terminated, since sexual reexposure then became possible. The mean duration of follow-up for the four-day tetracycline group was

20 days, compared with only 13 days for the seven-day treatment group. Of the men in the placebo group who were not re-treated with tetracycline, six were followed for 15 days and seven were followed for 20 days.

The 36 patients who did not respond to placebo were switched to either a four-day or a seven-day course of tetracycline. Of these, 29 were then followed for ten days or longer, and can be added to the group treated initially with tetracycline, in evaluating the effect of the duration of tetracycline treatment. At ten days and fifteen days after starting treatment, a greater proportion of patients treated with the four-day regimen manifested discharge with pyuria, or pyuria only, as compared with patients who were treated with the seven-day regimen (Table 3).

#### Discussion

Although most past studies<sup>1-5</sup> suggest a beneficial effect of tetracycline upon the course of NGU, the value of routine antibiotic treatment in NGU has been questioned by some workers.<sup>6,9,10</sup> Evaluation of these latter studies is difficult, because of the shortcomings mentioned earlier.

Table 4 summarizes several previous reports of tetracycline and placebo therapy. It is apparent that placebo-treated NGU tends to slowly subside over a period of one to three months in about 70% of cases. However, this is not especially comforting to the anxious patient. Furthermore complications such as epididymitis, prostatitis, and Reiter's syndrome do occur in a small percentage of untreated

patients with NGU.<sup>3,4,6</sup> The potential for transmission of untreated NGU should be recognized, although its transmissibility has never been demonstrated.

The response of NGU to tetracycline suggests that this syndrome is caused by one or perhaps more microbial agents. Antibiotic treatment, therefore, seems highly desirable if such treatment can be shown to abort the chronic, indolent course of placebo-treated NGU. The present short term investigation has shown that tetracycline is very significantly more effective than placebo in suppressing the symptoms and signs of NGU. Although other workers<sup>13</sup> found no advantage of a six-day course of a tetracycline over a three-day course, our results show that a seven-day regimen is more effective than a four day regimen in reducing the incidence of urethral inflammation within ten days, and that this effect is still evident at fifteen days, although the difference at fifteen days was no longer statistically significant. The major limitation of this study is the short length of the follow-up. It is not likely, however, that it would be possible to prevent sexual exposure and to follow 100% of the patients in a comparable treatment group at regular intervals for a much longer period of time. The longer follow-up in the four-day tetracycline group did not reveal additional recurrent failures at 20 or 25 days, even with this less effective regimen.

It is thought that the present findings form an adequate basis for the recommendation of a seven-day tetracycline regimen in the treatment of NGU. These findings do not preclude the possibility that continuing tetracycline for longer than seven days might be still more effective in the treatment of NGU.



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Table 1.--Diagnoses made in 206 men with symptoms of urethritis

Diagnosis	Total No.	Discharge with Pyuria	Pyuria Only
Acute Gonorrhoea	88	88	0
NGU	104	96*	8
No Objective Findings	14	0	0

\*Includes 20 patients who had been treated within previous six months for NGU, and 76 patients who had not been treated previously for NGU.

Table 2.--Number of patients treated for NGU and then retreated for persisting symptomatic urethritis, plus number with residual asymptomatic urethritis, after three different treatment regimens

	Total No.	Retreated	Asymptomatic Exudate or Pyuria at at Final Examination*	Total Failures
1) Tetracycline				
4 day	26	4 (15.4%)	5 (23.8%)**	9 (34.6%)
2) Tetracycline				
7 day	19	0	2 (10.5%)**	2 (10.5%)
3) Placebo				
4 day & 7 day	49	36 (73.5%)	6 (46.2%)**	42 (85.7%)

\*Of those patients not retreated, the mean duration of follow-up was 20 days for Group (1), 13 days for Group (2), and 18 days for Group (3).

\*\* % of remaining patients who were not retreated.

Table 3.--Comparison of 4 and 7 day tetracycline treatment in 74 men with NGU:  
 Number of men with persisting urethritis at 10 to 25 days after starting treatment.

Length of Tetracycline Treatment	No. men seen per <u>Day</u> of follow-up/ <u>No.</u> with discharge or pyuria and <u>%</u>											
	10 Days			15 Days			20 Days			25 Days		
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
4 Days	32	13*	40%	27	8	30%	19	3	16%	7	1	14%
7 Days	42	6	14%	20	2	10%	2	0	0	0	0	0

\*Includes 4 patients re-treated with erythromycin.

Patients with discharge or pyuria only at 20 and 25 days also had discharge or pyuria at 15 days,  
 and thus represent persistent NGU, not recurrent NGU.

Table 4.--Previously reported results of treatment of NGU  
with placebo or with a tetracycline at  
approximately seven day intervals after treatment

Treatment Used/Reference	7 days Post-Treat	Percent Failed 14 days Post-Treat	28 days Post-Treat
Placebo <sup>11</sup>			
(Potassium Citrate)	72.4	51.3	29.6
Placebo <sup>3</sup>			
(Potassium Citrate)	85	...	...
Placebo <sup>12</sup>	100	...	...
Placebo <sup>6</sup>	...	21	...
Terramycin <sup>1</sup>			
(250 mg 6 hourly for 6 days)	2	9	13
Terramycin <sup>3</sup>			
(250 mg 6 hourly for 4 days)	9	...	...
Tetracycline HCL <sup>2</sup>			
(250 mg 6 hourly for 6 days)	2	2	14

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CHAPTER V  
THE ROLE OF GONORRHEA AND OF MYCOPLASMA INFECTION  
IN CAUSING NONGONOCOCCAL URETHRITIS

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The opinions and assertions contained herein are those of the  
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views of the Navy Department or the Naval Service at large.



## Introduction

Over a period of about five weeks in April and May of 1966, it was possible to study an "epidemic" of urethritis which occurred aboard one of the U. S. Navy's largest aircraft carriers. The "epidemic" began when the ship left the mainland of the United States for a cruise in the Far East, and terminated when the ship returned to San Francisco seven months later. The "epidemic" was typical of those seen on all other major ships making ports of call in the Far East. During the cruise, the ship's 5,500 man crew was exposed to venereal infection during five different liberty periods in Subic Bay, R. P., and then was returned to sea for extended periods of time. This ship's operational schedule provided the nearest thing to an experimentally controlled situation. That is, one in which large numbers of men were exposed in one geographical area for short periods of time, and then were "confined" for observation. In such a unique setting, it was possible for an investigating team to board the ship for a five week period at sea. During this time the team performed clinical, epidemiologic, and laboratory studies on the entire spectrum of male urethritides, from gonococcal urethritis to "postgonococcal urethritis" to "nongonococcal urethritis."

The terms "nongonococcal urethritis" and "postgonococcal urethritis" may be confusing, unless they are closely defined. For the purpose of this discussion, nongonococcal urethritis (NGU) is defined as any case of urethritis in which urethral N. gonorrhoeae are absent but there is a demonstrable urethral exudate and/or pyuria of 20 or

more white blood cells per high-dry field in the first-glass urine sediment. First-glass pyuria of this magnitude has been shown to be indicative of urethritis.<sup>1</sup> Postgonococcal urethritis (PGU) is defined in this study as any case of urethritis which was no longer associated with the presence of demonstrable forms of N. gonorrhoeae, but which was preceded by typical N. gonorrhoeae infection of the urethra at some time during the seven month cruise. Thus PGU actually comprises a certain fraction of the NGU syndrome, as will be discussed subsequently.

In previous papers,<sup>1-3</sup> we have presented data obtained from this five-week study pertaining to certain aspects of the epidemiology and treatment of gonococcal and postgonococcal urethritis, and to the treatment of nongonococcal urethritis. The present paper is addressed to the more elusive problems of the epidemiology and etiology of nongonococcal urethritis. Particular emphasis is placed upon the apparent relationship of NGU to PGU.

In addition, the results of studies of the prevalence of genitourinary mycoplasma infection in various groups of individuals are analyzed. A comparison was made of the prevalence of large colony and T-strain mycoplasmas in men with gonorrhoea or with NGU, in healthy male controls, and in female prostitutes from the Subic Bay area. Patients and controls were interviewed regarding history of sexual contacts, to determine the influence of intercourse upon the presence of urethral mycoplasmas. Men with gonorrhoea or NGU were cultured for urethral mycoplasmas before and after antibiotic treatment, and the persistence of urethral mycoplasmas was studied in relation to the response to treatment. Finally, large colony mycoplasma isolated were identified

according to serotype, and an attempt was made to propagate T-strain isolates.

Study Population.--For the most part, the study population has previously been described.<sup>1-3</sup> Male patients were members of the 5,500 man crew of an aircraft carrier. Only patients with symptoms of urethritis who reported to sick call after the ship left Subic Bay on April 20, 1966, and before the ship returned to Subic Bay on May 18, 1966, were included in the study. Another 75 men without genitourinary symptoms were selected from the daily sick call line as controls for the mycoplasma studies.

Females who were examined for genitourinary mycoplasma were employed as hostesses in the town of Olongapo, which borders the Navy's Subic Bay complex. Approximately 5,000 hostesses are registered with the municipal health department and are required to report to a social hygiene clinic weekly for a pelvic examination and cervical culture for N. gonorrhoeae. From this group, 25 hostesses were examined and swabs of the vaginal vault and cervical os were taken for mycoplasma culture.

Laboratory Studies.--All male patients with urethritis were interviewed and several specimens of urethral exudate and scrapings were obtained and processed for bacterial, viral, and mycoplasma studies, as previously reported.<sup>1,2</sup> A diagnosis of gonorrhea was made if N. gonorrhoeae was isolated from the urethra. A diagnosis of NGU was made if a urethral exudate or pyuria (20 or more WBC/HDF in the first-glass urinalysis) was demonstrated, but three successive daily examinations did not reveal N. gonorrhoeae.

Urethral scrapings from controls and men with gonorrhoea or NGU were frozen for future viral studies.

Treatment and follow-up.--Gonorrhoea patients were treated with one dose of 2.4 million units of procaine penicillin G plus oral probenecid, or with 9.5 grams of oral tetracycline hydrochloride over a four day period.<sup>2</sup> Men with NGU received either placebo or the same four day oral tetracycline regimen (some received a similar seven day tetracycline regimen) in a double-blind, semi-crossover study.<sup>3</sup> All patients were reexamined and urine specimens were obtained regularly at five day intervals until the ship reentered port. A second specimen of urethral scrapings for mycoplasma culture was obtained five days after starting treatment from all men with gonorrhoea or NGU. Second specimens were not obtained from the male controls or from hostesses.

Only one patient with gonorrhoea had persistence of urethral N. gonorrhoeae after treatment, and he was not included in this study. Of the remaining gonorrhoea patients, those who still had a demonstrable urethral exudate or pyuria alone 20 days after treatment was started were considered to have PGU.

Similarly men with NGU who had persistence of urethral exudate or pyuria after placebo or tetracycline were defined as treatment failures. The duration of follow-up was less than 20 days in most patients with NGU, since the onset of symptoms usually occurred later than in men with gonorrhoea, and the study was terminated when the ship reentered Subic Bay.

## Results

Incidence of Gonorrhoea and NGU.--During seven months of the cruise, from the time the ship left the U. S. through the completion of the present study, the crew experienced a total of 454 cases of acute gonorrhoea and 246 new cases of nongonococcal urethritis. Thus, NGU accounted for about one-third of the 700 individual cases of urethritis seen by the ship's medical department during the cruise. During this time gonorrhoea was diagnosed by bacterial culture, and the usual treatment given for gonorrhoea was 2.4 million units of procaine penicillin G, sometimes in combination with 1.2 million units of benzathine penicillin G. The proportion of NGU was higher during the last five weeks, when the investigating team was aboard the ship. During this period a total of 88 cases of acute gonococcal urethritis and 104 cases of NGU were seen. Of the NGU cases, 70 occurred in men who were previously untreated and who first developed symptoms of urethritis during or after the liberty period which preceded the onset of the study. This apparently reflected an increasing proportion of NGU toward the end of the cruise. A retrospective review of medical records showed that after the first two liberty periods, 29% of all urethritides were NGU; after the third and fourth liberties 36% were NGU, and after the fifth liberty period 70 of 158 or 44% of newly occurring urethritides were NGU. In those men with gonorrhoea, the infection followed a typically acute course with a brief incubation period. The peak incidence of onset occurred on the sixth and last day of liberty, and 85 of 88 cases had become symptomatic within 6 days of leaving port (Figure 1). In contrast, NGU was clinically a more indolent infection and the

daily incidence rose gradually during the first several days of the study, and then showed a gradual decline with considerable fluctuation. New cases were still appearing when it was decided to stop admitting new cases of NGU to the study as the opportunity for sufficient post-treatment follow-up became limited. It is probable that even more than 70 new cases of NGU would have been found had the input of NGU cases been permitted to continue.

Relationship of NGU to PGU.--Despite the successful eradication of demonstrable N. gonorrhoeae from 58 men treated with 2.4 million units of procaine penicillin G plus probenecid, it was found that three weeks after treatment two-thirds of these men had developed objective signs of post gonococcal urethritis (PGU.)<sup>1</sup> Only 16 of them, or slightly over 20% of the total penicillin-probenecid treatment group, actually had symptomatic PGU. It is quite clear that men with symptomatic PGU were indistinguishable from men with the NGU syndrome, and were, in fact, receiving the diagnosis of NGU and being treated accordingly by the ship's medical department. A typical case report serves to illustrate this point: K. V., a 20 year old Caucasian petty officer, developed symptoms of urethritis on 12/3/65, three days after the ship put out to sea following a four-day liberty in Subic Bay. A culture of urethral exudate revealed organisms typical of N. gonorrhoeae. He was treated with a single IM injection of 2.4 million units of procaine penicillin G. A week later, on 12/10/65, a urinalysis was "essentially negative." On 12/12/65 he developed recurrent dysuria and urethral exudate, and a repeat culture grew only "staphylococci." On 12/15/65 a diagnosis of NGU was made and he was started on Furadantin, 100 mg four times a day for

seven days. On 12/23/65, because of persistent symptoms and signs of urethritis he was begun on Pyridium in an unspecified dosage. On 1/1/66 and 1/14/67, while the ship was still at sea, he continued to manifest urethral exudate on follow-up examinations. On 1/16/67 a culture of the urethral exudate on chocolate agar medium showed no growth, and he was treated with chlortetracycline, 500 mg four times a day for seven days. His exudate and symptoms then disappeared except for an itching sensation during urination which persisted for the next 100 days. On 4/25/67 he again noted a urethral exudate. Examination at that time by the investigating team revealed a slight, clear urethral exudate, and the first-glass urine sediment showed 30 to 40 WBC per high-dry field. N. gonorrhoeae was not recovered in multiple cultures. He was considered to have NGU and was treated with tetracycline, 1500 mg initially followed by 500 mg every six hours for 4 days. Follow-up examination over the next 20 days showed a complete disappearance of symptoms, exudate, and pyuria.

Thus, it is apparent that PGU can be distinguished from NGU (if, indeed, such a distinction is to be made) only by the history of recent gonorrhea in the former group. It can be calculated that of the 454 gonorrhea cases encountered during the seven month cruise, 20% or approximately 90 men should have developed symptomatic PGU. It was not possible to retrospectively analyze all of the cases of symptomatic NGU seen during the cruise to determine what proportion had a recent history of gonorrhea. Such an analysis was possible, however, in the group of 70 men who had their onset of NGU after April 14. Only 11% of these men had had gonorrhea during the cruise (Table 1). This incidence was only slightly greater than the 8% incidence in controls. However, patients

who developed symptomatic PGU during the investigation, and who would have been diagnosed and treated by the ship's medical department as NGU prior to the study, were not included in the NGU group, since they were already in the gonorrhea follow-up group. If the 16 patients who developed symptomatic PGU are combined with the 70 patients with NGU, then 28% of the total of 86 patients had had gonorrhea at sometime during the cruise.

Results of Mycoplasma Studies.--The results of cultures for mycoplasmas from 75 health male controls, 104 men with NGU, 88 men with gonorrhea, and 25 randomly selected female hostesses are summarized in Table 2. From the column which lists the initial isolation percentage, it is apparent that the mycoplasmas were more commonly isolated in the control and NGU groups from men who admitted recent sexual contact than from those who denied recent contact. When the control and NGU groups are combined (Table 3), the percentage of isolation from men with recent sexual contact is significantly greater than from men without recent contact ( $P < .05$ ). This finding is particularly significant since it is probable that several of the controls and the patients with NGU who denied contact during the previous liberty period nonetheless did have sexual contact earlier in the cruise.

Referring again to Table 2, the highest initial isolation percentage (35%) was from patients with NGU who admitted sexual contact during the previous liberty period in Subic Bay, while the lowest isolation rate was from controls who denied recent sexual contact (14%). The overall initial isolation rate from patients with NGU was 32.1%, compared with 22.7% in all controls. This difference was not statistically significant.



However, the data suggest that mycoplasmas are found more often in patients with NGU than in normal controls or men with acute gonorrhoea, even when the groups are matched according to sexual activity.

As previously described,<sup>2</sup> patients with gonorrhoea were treated either with a single injection of procaine penicillin G plus oral probenecid, or with oral tetracycline; and patients with NGU were treated either with a lactose placebo or with oral tetracycline.<sup>3</sup> Since specimens for mycoplasma isolation were obtained from all patients before treatment and again five days later, it was possible to study the effects of these regimens upon the recovery of urethral mycoplasmas. It can be seen in Table 4 that the penicillin-probenecid and placebo regimens had no apparent influence on the mycoplasma flora, whereas mycoplasmas were recoverable in the second specimens from only 6 of 25 patients with mycoplasma-associated urethritis who were treated with tetracycline. It is of interest that all six patients had received a four day tetracycline regimen (1500 mg initially followed by 500 mg every six hours for four days). Five of the 25 patients treated with tetracycline received the antibiotic for seven days. Two of the five were recultured the second time on the sixth day of treatment, and three were recultured three days after completion of treatment, and persisting mycoplasmas were not found in any of these men. It may be, therefore, that the longer regimen is more effective in eradicating urethral mycoplasmas, but follow-up cultures would have to be done in several more patients at longer intervals after a seven day course of tetracycline in order to prove this point.

A particularly interesting finding (Table 5) was the relationship

of the persistence of mycoplasmas in the urethra to the failure of NGU to improve with tetracycline. Of seven patients with mycoplasma-associated NGU who failed to improve on the four day tetracycline regimen, five had persisting urethral mycoplasma; whereas, of ten patients with mycoplasma-associated NGU who were cured with tetracycline, only one had persisting mycoplasma in the post-treatment culture. This is a significant difference ( $P < .05$ ) which suggests that the persistence of mycoplasma is related to the persistence of urethritis.

The mycoplasmas which were isolated from the four study groups listed in Table 1 were separable into two categories on the basis of colonial morphology (Table 6). Two-thirds of the isolates formed typical large colonies on the isolation plate. Of 60 large colony isolates which were transported to the laboratory in Honolulu and successfully propagated, 59 were serotyped as Mycoplasma hominis by the method previously described.<sup>1</sup> The remaining one-third of the isolates formed very small colonies which were less than 25 microns in diameter and were found only by the use of Diene's staining procedure.<sup>4</sup> These small colonies apparently represent the "T-strain" mycoplasmas described by Shepard,<sup>5</sup> and found by Ford<sup>6</sup> in up to 79% of men with NGU. The latter author used a culture medium and an isolation technique which were essentially the same as were used in the present study. However, it is noteworthy that 38 of 47, or 81% of the "T-strains" recovered in the present study were isolated in association with large colony M. hominis. Usually the T-strains were seen in a satellite position near the periphery of a large colony. It was not possible to successfully propagate any of the T-strains beyond primary culture. In several instances,

subcultures of satellite T-strains appeared to revert to large colony forms, but when T-strain colonies were picked from the primary plates, contamination from adjacent large colonies may have occurred.

## Discussion

The etiology of nongonococcal urethritis in men remains obscure despite years of intensive study. The opportunity to approach the problem on a large aircraft carrier was unique, since a homogeneous group of young men were periodically exposed to a well studied population of female prostitutes for short periods of time, and were then confined aboard the ship while it was at sea. During this latter period, heterosexual reexposure was not possible, and the population was easily controlled as evidenced by virtually 100% attendance of urethritis patients at over 650 scheduled follow-up examinations.

No attempt was made to employ routine culture media in comparing the bacterial flora of the urethra of controls with the flora of patients with NGU, since well controlled studies had already shown that essentially no difference exists.<sup>7,8</sup> Previous attempts<sup>9</sup> to demonstrate an association of NGU with anaerobic bacterial infection had also been unsuccessful, and anaerobic bacterial cultures were not performed in this investigation. A small proportion of NGU in men appears to be associated with the presence of urethral *Trichomonas* species,<sup>10</sup> but trichomonads were not observed in any of the urinalyses performed on these men.

Although inclusion bodies have been seen in Giemsa-stained urethral scrapings from men with NGU,<sup>11,12</sup> Willcox<sup>8</sup> examined 1,463 specimens and concluded that these inclusion bodies were not concerned with the causation of NGU. Attempts to isolate viral agents, employing various types of tissue culture and chick embryos have generally been unrewarding,<sup>13</sup> but agents of the TRIC group have been isolated from a

few men with NGU.<sup>14</sup> Urethral scraping specimens were obtained from patients and controls for future viral studies. These studies will be carried out at the U. S. Army 406th Laboratory, Camp Zama, Japan.

One of the most unique aspects of the shipboard investigation was the opportunity to study the relationship of NGU with gonococcal urethritis. Temporally, gonorrhoea preceded NGU. As shown in figure 1, many cases of NGU continued to develop after clinical gonococcal urethritis had disappeared. This lag in development of NGU over the short term may correspond to the apparent long term lag in development of NGU, which was suggested by the relatively greater proportion of nongonococcal urethritis during the latter three months of the cruise. This did not reflect an accumulation of unsuccessfully treated cases of NGU, since only newly diagnosed cases of NGU were included in these computations.

It may be that the short term and long term incidence curves of gonorrhoea and of NGU represent independently occurring phenomena. However, in view of the occurrence of PGU in two-thirds of penicillin-treated gonorrhoea patients, one suspects that each burst of gonorrhoea infections following a liberty period left its "tail" of chronic post-gonococcal infections, similar to the case described earlier. A gradual accumulation of such cases could contribute to the long term rise in incidence of NGU, relative to gonorrhoea.

Sixteen patients with symptomatic PGU were grouped together with the 70 patients with newly diagnosed NGU, in order to arrive at an estimate that 28% of cases of NGU were preceded at some point during the cruise by acute gonorrhoea. This manipulation of data was at least partially justified by the fact that earlier in the cruise patients who

developed symptomatic PGU did seek treatment and were regarded by the ship's medical department as having NGU. Furthermore, this approach to PGU has been encountered on other large ships<sup>1</sup> and is thought to be a standard medical practice throughout the Navy. The estimate at best is an approximation of the proportion of nongonococcal urethritides which are actually PGU, and would vary according to the type of treatment and follow-up examination employed in the management of gonorrhoea.

It is possible that a greater proportion of the NGU cases were preceded by gonorrhoea, since antibiotics were readily available in the town of Olongapo and some men may have attempted self-treatment rather than submit to medical quarantine while the ship was in port. This would have entailed confinement to the ship during treatment by the ship's medical department. However, in confidential interviews, self-treatment and prophylactic antibiotic treatment were denied by all patients with gonorrhoea or NGU, and by all of the controls.

Finally, as a matter of speculation, it is possible that some cases of NGU actually presented infection by a variant of N. gonorrhoeae which cannot be detected by routine methods. As previously discussed,<sup>1</sup> there are some data which suggest that gonococcal L-forms may be involved in the chronic urethritis which follows acute gonorrhoea after penicillin treatment (PGU). Recently, chronic gonorrhoea infection of the male has also been described.<sup>15</sup> Certainly not all men who are exposed to the infected female with gonorrhoea develop acute gonorrhoea.<sup>16</sup> Questions arise as to whether subclinical gonococcal urethritis occurs in the male, and whether variant forms of N. gonorrhoeae may be involved.

Prior to World War II, the opinion was widely held that NGU represented a chronic form of gonorrhoea. In the absence of any supporting evidence, this view became untenable. Now the pendulum appears to be swinging back, and the possibility that some cases of NGU are caused by occult infection by variant forms of N. gonorrhoeae must be seriously considered.

Gonorrhoea may also lead to chronic urethritis simply by injuring the urethral mucosa and permitting secondary invasion by certain urethral commensals. In the previous paper<sup>1</sup> we presented data showing a highly significant association of the presence of mycoplasmas in the urethra with the development of postgonococcal urethritis after treatment with penicillin and probenecid. Because of the apparent relationship between PGU and NGU it was of interest to explore the role of urethral mycoplasma infection in causing NGU. It is apparent in Table 3 that fewer than one-half of all cases of NGU were associated with the presence of mycoplasmas in the urethra. Therefore, the essential question apparently is not whether all cases of NGU are caused by mycoplasmas; rather, the question is whether, in mycoplasma-associated NGU, the mycoplasmas are causal or are just commensals which are coincidentally present. In this respect the association of the failure to improve after tetracycline with persistence of urethral mycoplasma infection suggests that the mycoplasmas are indeed causal.

In Table 5, the five unsuccessfully treated patients with persistent mycoplasma, all carried a strain of M. hominis. Although some other workers have found T-strains to be more commonly associated with NGU than large colony mycoplasmas,<sup>6,17,18</sup> we observed the opposite (Table 6). Our failure to isolate T-strains more frequently than large colony

mycoplasmas from patients with NGU may be due to differences in technique, differences in the population studied, or even due to geographic differences. The possibility that T-strain mycoplasma comprise a distinct taxonomic species which is involved in the causation of some cases of NGU is certainly not discounted by the present findings. However, the frequent occurrence of these T-strain colonies as satellites around larger colonies and the finding of temperature sensitivity in two different successfully propagated T-strains which we have studied,<sup>19</sup> suggests that in some cases these may simply arise as genetic variants of the large colonies. The recent observation<sup>20</sup> that the T-strains form an antigenically heterogeneous group is consistent with this concept, since M. hominis is a uniquely antigenically heterogeneous species.<sup>21</sup> For these reasons we have considered the T-strains and the large colony mycoplasmas from the urethra together as a group in evaluating their role in NGU. If mycoplasmas are indeed causal in some cases of NGU, there is a seeming inconsistency in the high prevalence of urethral mycoplasmas in asymptomatic controls. Much of the controversy in the literature regarding the role of mycoplasmas in NGU has centered around this point. In controlled studies, reviewed by Hayflick and Chanock,<sup>22</sup> some workers have isolated mycoplasmas from the urethra more often from patients with NGU than from controls, while others have observed no difference. Our data indicates a higher prevalence in patients with NGU than in controls or in men with gonorrhoea, even when the groups are matched according to sexual activity, but the difference was not statistically significant.

This point is not critical, however, if it is assumed that



mycoplasmas produce clinical urethritis in only a small proportion of men who are "carriers" of urethral mycoplasmas. This sort of situation is characteristic of mycoplasma infections,<sup>23,25</sup> and of many bacterial infections, such as streptococcal pharyngitis or meningococcal meningitis, which occur in only a small percentage of carriers of Group A streptococcus or of N. meningitides.<sup>26,27</sup> Those factors which lower the host's resistance and permit pathogenic expression of mycoplasma infection would include but not necessarily be limited to gonococcal infection itself.

In this sense, mycoplasmas might be regarded as members of the normal urethral flora in most individuals who harbor these organisms. However, the mycoplasmas differ from the other commensals in one important respect. As illustrated in Table 3, the probability of isolating a strain of mycoplasma from the urethra was significantly greater in men who admitted recent sexual exposure to prostitutes than in men who denied exposure. The opinion is widely held that NGU is a venereal disease.<sup>8</sup> The incidence of NGU in the ship's crew was nearly 5% in only seven months, which is obviously higher than the incidence in non-promiscuous, married men whose sexual relations are limited to their marital partners. One is led to look for a causal microbial agent which is more prevalent in the genital tract of promiscuous women than in healthy married women. Previous studies have shown that mycoplasmas are far less prevalent in healthy women or private gynecology patients than in women with inflammatory pelvic disease or prostitutes.<sup>6,28</sup> In the present study, 72% of the hostesses examined harbored mycoplasma in the vagina and cervix.

Thus, the hypothesis is advanced that genital mycoplasma infection

is highly prevalent among female prostitutes in Olongapo; that men acquire mycoplasma infection of the urethra by sexual contact with these prostitutes; and that the mycoplasmas produce nongonococcal urethritis in a small percentage of these men, often as a direct consequence of urethral mucosal trauma produced by gonorrhoea itself.

Thus far this hypothesis has not been substantiated by successful reproduction of NGU in a susceptible host with strains of mycoplasma which have been isolated from the urethra and serially propagated in vitro. Oddly enough, it has been possible to experimentally produce an exudative pharyngitis by inoculating human volunteers with M. hominis,<sup>29</sup> but associative analyses have failed to show any relationship of M. hominis to naturally occurring pharyngitis.<sup>30,31</sup> On the other hand, the etiologies of many infectious diseases have been determined with reasonable certainty without fulfilling all of Koch's postulates - usually through the associative type of analysis used in the present study. The classic example is leprosy, but the various infections produced by secondary pathogens are often in this category.

The proposed role of mycoplasma infection of the urethra in causing NGU appears to be a particularly interesting example of secondary pathogenesis because it is possible to define the mode of acquisition of infection; and because one supposed predisposing "stress" which reduces the host's resistance to secondary infection (i.e. gonococcal urethritis) is both naturally occurring and frequently encountered.

Table 1. Proportion with history of gonorrhoea during cruise among three groups of men: controls; previously untreated NGU; and NGU combined with symptomatic PGU

Patient Group	Total No.	No. with GC in Past 6 Mos.	Per Cent
Controls	75	6	8%
Untreated NGU	70	8	11%
NGU and PGU Combined	86	24	28%

Table 2. Isolation of mycoplasma from healthy male controls, men with NGU or gonorrhea, and female hostesses. Control and NGU groups are subdivided according to history of sexual contact during the previous liberty period

Diagnosis	No. of Individuals	Initial No.	Isolates %	Total No.	Isolates* %
Control - Admitted Contact	40	12	30	--	--
- Denied Contact	35	5	14	--	--
NGU - Admitted Contact	68	24	35	30	44
- Denied Contact	36	10	28	11	30
Gonorrhea	88	19	22	25	28
Hostess	25	18	72	--	--

\*Includes initial isolates from all groups plus post-treatment isolates from specimens collected from NGU and gonorrhea groups only.

Table 3. The relationship of recent sexual contact to the isolation of urethral mycoplasmas in 106 NGU patients and 75 controls

Study Group	Recent Sex Contact		Number Harboring Mycoplasma	Probability
	Yes	No		
NGU Patients	68		30 (44%)	P= >.05
		36	11 (31%)	
Controls	40		12 (30%)	P= >.05
		35	5 (14%)	
Combined Total	108		42 (39%)	P= <.05
		71	16 (23%)	

Table 4. Recovery of urethral mycoplasmas from men with urethritis (NGU or gonorrhea) before treatment and five days after starting treatment with tetracycline, penicillin-probenecid, or placebo

Mycoplasma Isolated	Tetracycline	Penicillin-Probenecid	Placebo
Pre-treatment only	19	5	3
Post-treatment only	1*	6	4
Both	5*	6	10

\*Mycoplasma were recovered from six patients following the four-day tetracycline regimen, but were not recovered from patients on the seven-day tetracycline regimen.

Table 5. Relationship of tetracycline treatment failure to persistence of urethral mycoplasma in post-treatment cultures from patients with mycoplasma-associated NGU

Clinical Response	Total No. Individuals	No. with Persisting Mycoplasma	% with Persisting Mycoplasma
Failures	7	5	71%
Cures	10	1	10%

P < .05

Table 6. Relative frequency of isolation of large colony\* and T-strain mycoplasmas from four study groups

Catetory	No. Patients Studied	Large Colony* only	T-strain only	Both
NGU	104	21	4	18
Gonorrhoea	88	12	3	10
Control	75	10	1	6
Hostess	25	14	1	3
Total	292	57 (19.5%)	9 (3.8%)	37 (12.7%)

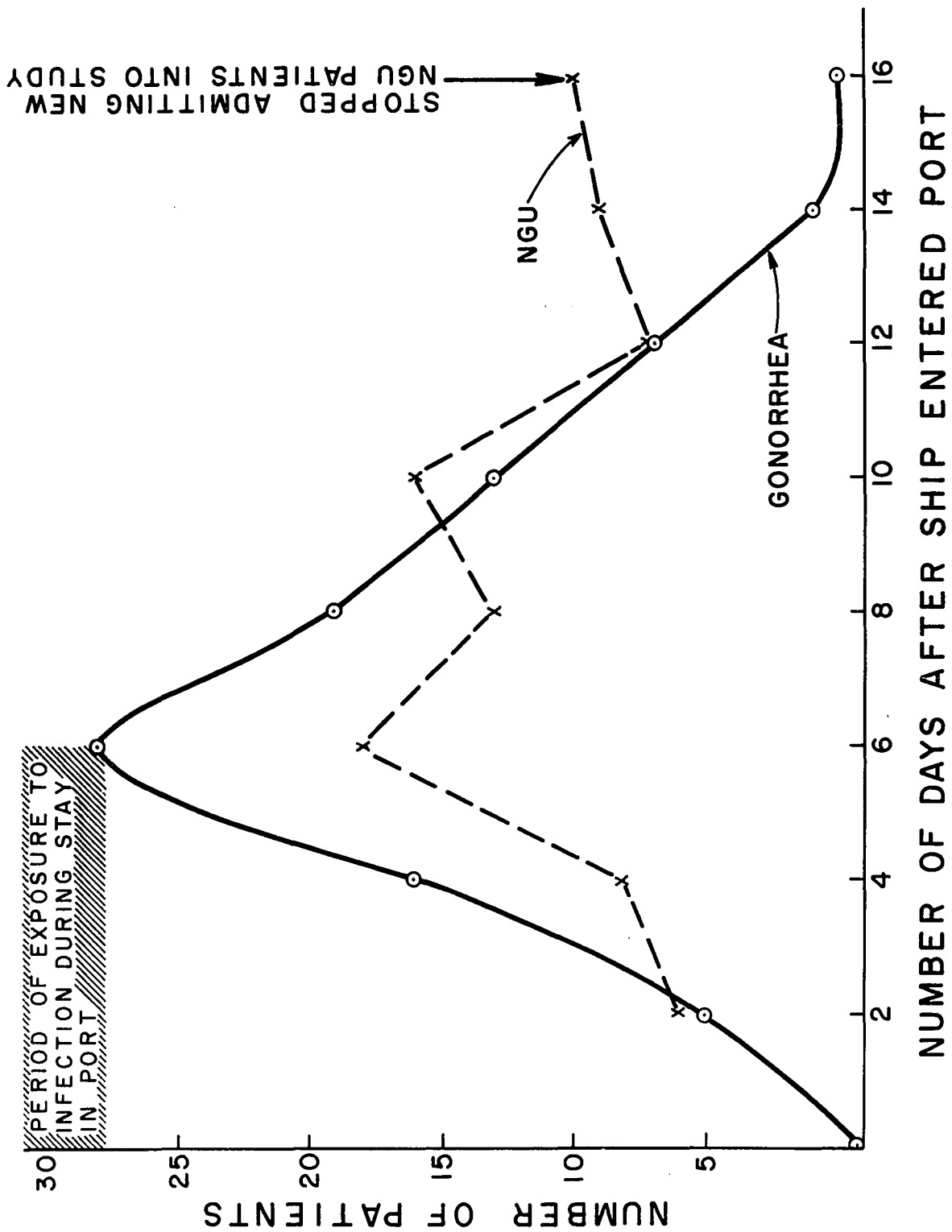
\*Of 60 large colony strains successfully transported to the laboratory and propagated, 59 were identified as M. hominis and one has not been serotypable.



The Role of Gonorrhea and of Mycoplasma Infection in Causing  
Nongonococcal Urethritis.

LT King K. Holmes, MC, USNR

Legend for Figure 1.--Time of onset of symptoms of urethritis, as  
determined by interview of 88 men with gonorrhea and 70 men with NGU.



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CHAPTER VI  
SUMMARY AND PERSPECTIVES

1. The evaluation of various treatment regimens for treatment of gonococcal urethritis in men demonstrated two therapeutic principles. The first was that adequate treatment of gonorrhoea in the male depends upon the serum concentration of antibiotic. The second was that it is not necessary to maintain bacteriostatic levels in the serum for 48 hours to ensure eradication of N. gonorrhoeae from the urethra, as previously believed, if the peak concentration of penicillin reached in the serum is sufficiently high. We have unpublished data which show that the mean peak serum concentration attained with the penicillin-probenecid regimen used in this study was about 30 U per ml and that the level had fallen below the M.I.C. of most strains of N. gonorrhoeae within 24 hours. In vitro studies are now in progress to analyze more closely the effect upon Neisseria of brief exposures to very high concentrations of penicillin. The apparent effectiveness of such treatment in gonorrhoea may have applicability to infections caused by bacteria other than Neisseria.

2. The penicillin-probenecid and tetracycline regimens described in chapter one have been widely adopted by Navy physicians in the Far East, and the penicillin-probenecid regimen has recently been recommended by the U. S. Public Health Service for treatment of "penicillin-resistant" gonorrhoea. In a cooperative study now being carried out aboard eight aircraft carriers and several other large Navy vessels, the effectiveness of these regimens has been substantiated in well over 1,000 patients with gonorrhoea.

3. One of the most interesting findings in these studies was the

high incidence of PGU following penicillin treatment of gonorrhoea. The only evidence presented here for the suggested role of gonococcal L-forms in causing PGU was the data relating to PGU to the initial penicillin resistance of the infecting strain of N. gonorrhoeae. Nonetheless, we believe this is a most promising avenue of future investigation. We have recently succeeded in inducing and propagating L-forms of N. gonorrhoeae in our laboratory, and are now developing a protocol for an attempt to isolate L-forms from patients who have been treated for gonorrhoea with penicillin. The survival curve presented in chapter one will be useful in planning the study.

Additional information regarding the possible role of penicillin-induced variants of N. gonorrhoeae in causing NGU will be sought rather indirectly. In June 1967, it is planned to institute a program of mass prophylaxis in the Olongapo prostitutes. Bristol Laboratories have made available 35,000 capsules ampicillin, which will permit administration of seven capsules to as many of the 4,800 registered hostesses as can be rounded up. This dosage has been established as virtually 100% "curative" in these women. It will be possible to follow the relative incidence of gonorrhoea and NGU in Navy personnel exposed to these women before and after the prophylaxis. If the incidence of NGU is unaffected, while the gonorrhoea incidence falls, this would suggest that the two diseases occur independently. If the incidence of both fall, it would suggest that the prevention of gonorrhoea in the male was sufficient to also prevent NGU in the male. If the incidence of NGU increases while the incidence of gonorrhoea falls, it might suggest that the increase of NGU was due to the induction of gonococcal variants

in the women by the prophylaxis program.

4. The proposed role of mycoplasmas in causing NGU is interesting from the standpoint of host-parasite relationships as an example of secondary pathogenecity. Equally intriguing at the moment is the relationship of the T-strain mycoplasmas to the classicial large colony mycoplasmas. It is hoped that immunologic studies being done at NIH by Purcell; taxonomic-physiology studies being done by D. E. Wright at the Navy Medical Research Unit No. 1 in Oakland; and our own study of the temperature-sensitivity of these organisms will help to clarify this relationship in the near future.