

Studies on the Susceptibility of Gibbons to Gonococcal Infection

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OBJECTIVE: To determine whether the white-handed gibbon (*Hylobates lar*) would serve as a satisfactory host for experimental infection with *Neisseria gonorrhoeae*.

BACKGROUND: Attempts to produce experimental gonococcal infection in common laboratory animals have invariably failed in the past (1, 2). Varying degrees of success have been reported in infecting rabbits by inoculating *N. gonorrhoeae* into the anterior chamber of the eye (3) and by culturing the organism in a golf ball subcutaneously implanted into the rabbit (1). Experimental gonococcal urethritis has been successfully produced by inoculating pus from gonorrhea patients (4) or Type 1 gonococcal suspension grown *in vitro* into the chimpanzee intra-urethrally (5). Gonococcal urethritis in chimpanzees resembles urethritis which occurs in humans. The gibbon is often used as an experimental animal in the study of diseases of humans; therefore, it was deemed practical to attempt an experimental infection with *N. gonorrhoeae*. The study was divided into four parts.

PART I: Cellular Components of Gonococcal Urethral Exudate of Male Patients.

INTRODUCTION: Gonococcal urethral exudates of male patients have been widely used as inocula to produce experimental gonorrhea both in human volunteers and in laboratory animals. A study on the phagocytic activity, the cellular composition, and the concentration of viable gonococci in acute gonorrheal exudates was done.

DESCRIPTION: Male patients with urethritis who had intracellular gram negative diplococci in the urethral exudate smear were selected for this study. From each patient, a urethral exudate smear was prepared and a small amount of the exudate was transferred by means of a wire loop into a sterile centrifuge tube containing 1 ml of GCBID medium (GC broth supplemented with 1% Isovitalax and 10% defined supplement of Dr. D.S. Kellogg). The gram stained smears were examined and phagocytosis and extracellular gonococci quantitated.

Phagocytic activity was defined as the per cent of 400 polymorphonuclear leukocytes (PMN) which contained gram negative diplococci, morphologically typical of gonococci and in the focal plane of the PMN. Extracellular gonococci were recorded as the per cent of 500 gonococci distributed randomly in the smear both intracellularly and extracellularly. A differential white blood cell count was performed on 100 WBC per smear. The number of viable gonococci present in the exudate was determined by enumerating colonies on GCBID agar after incubation at 36°C.

RESULTS: Results obtained from the urethral exudates of 12 Thai male patients are given in Table 1. The amount of urethral discharge obtained from each patient was small and variable. The exact quantity of each exudate sample was not measured.

DISCUSSION: The results indicate that almost all gonococci contained in the acute urethral discharge are intracellular. Of the 12 specimens studied, extracellular gonococci ranged from 0 to 15% with an average

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of 6%. The number of viable organisms varied from 2.4×10^5 to 3.6×10^6 per patient sample. Such variation may have been due in large part to variation in the amount of exudate collected from each patient. Average per cent PMN among the specimens was 97% with a narrow range of distribution from 93 to 98%. Phagocytic activities of these PMN varied from 2 to 51% with an average of 25%. Such variation in the phagocytic activity of PMN is not unexpected, and agrees well with *in vitro* results reported elsewhere. The study failed to show any correlation between the percentage of extracellular gonococci and the number of viable cells in the exudate.

PART II: Effect of in vitro Incubation at Higher Temperature (39°C) on Type I Gonococci.

INTRODUCTION: Inoculation of virulent gonococci, either as a suspension of pure culture, or as purulent exudate obtained directly from patients, into sub-human primates, e.g., the gibbon, consistently failed to produce gonococcal infection (6). Gonococci grow optimally at 36°C; therefore, the failure of gonococci to grow and establish infection in the gibbon might be due to the sensitivity of gonococci to higher temperature. Because the normal body temperature of the gibbon is 39°C, we wished to determine the effect of long exposure at this temperature on gonococci.

DESCRIPTION: Both *N. gonorrhoeae* F62 Type 1 in pure culture and Type I gonococci contained in urethral exudate obtained from acute gonorrhea patients were used in this study. The gonococcal suspension was prepared and diluted with GCBID medium. Triplicate samples of each dilution were incubated both at 36°C and 39°C. Incubation was carried out for 48 hrs before determining viability.

RESULTS: Figure 1 shows the viability of gonococci at 39°C compared to that at 36°C. The total viable gonococcal colonies counted after 48 hrs of incubation at 36°C was taken as 100% viability, and viability of gonococci at 39°C was expressed as per cent of viability at 36°C.

In all 4 specimens, viability of urethral exudative gonococci at 39°C was reduced, e.g., 0.01, 85.3, 63, and 33 per cent of their corresponding viability at 36°C. Viability of *N. gonorrhoeae* F62 in pure culture was unaffected by incubation at the raised temperature. Colonial typing was performed after 24 hrs of incubation. No morphological differences were observed in colonies produced by gonococci in pure cultures or gonococci in exudate after incubation at 36°C or 39°C.

DISCUSSION: Type I gonococci in the urethral exudates demonstrated loss of viability after incubation at 39°C. While there was nearly total loss of viability in one exudate, there was no significant change in two other exudates. Conversely, gonococci in pure culture (F62 Type I) survived incubation at 39°C. It is tempting to associate the sensitivity of exudative gonococci to incubation at high temperature with their exposure to the PMN which were present in abundance in the urethral exudates. In this regard the relatively higher phagocytic rate in the 2 affected exudative specimens (Table 2) supports this hypothesis; however, direct studies on the phagocytic and bactericidal effect of exudate on gonococci is needed to establish the point.

Although the data are limited, the results indicate that *in vitro* incubation at 39°C for 48 hrs does not adversely affect virulent gonococci in pure culture either in viability or in colonial type. Less conclusive is the effect of incubation at 39°C for 48 hrs on exudative gonococci from patients. The evidence indicates that gonococci in urethral exudate have reduced viability after incubation at 39°C; phagocytosis may be responsible.

PART III: In vitro Phagocytic and Bactericidal Activity of Gibbon Leukocytes on Virulent Gonococci (F62).

INTRODUCTION: As an alternative to human volunteers, subhuman primates have been used as close-to-man models for gonococcal infections, both successfully, in the case of chimpanzees (4), and unsuccessfully, in the case of gibbons (6). Since the susceptibility of man to gonococcal infections has been attributed to the relatively low bactericidal activity of his leukocytes compared to that of lower animals, it was necessary to define the interaction of gibbon leukocytes and the virulent gonococci.

Table 1. Cellular Compositions of Smears Prepared from Gonorrheal Urethral Exudates of Male Patients.

Patient No.	Gonococci		Leukocytes	
	Extracellular (Per Cent)	Viable Counts Per Sample X 10 ⁵	PMN Per Cent	Phagocytosis Per Cent
1	13	4.4	96	28
2	4	2.4	98	23
3	3	5.6	98	51
4	7	3.5	98	6
5	1	9.8	98	2
6	15	13.0	93	26
7	11	16.0	98	27
8	0	5.1	96	29
9	1	46.0	95	49
10	8	2.8	97	13
11	2	6.4	98	19
12	8	13.0	95	32
Average	6	14.8	97	25

Table 2. Viability of *N. gonorrhoeae* from Urethral Exudates and *in vitro* Culture Suspensions of Strain F62.

Exudate No.	Loss of Viability at 39°C (per cent of Viability at 36°C)	Phagocytic activity* (per cent)
1	99.9	51
2	14.7	6
3	37.0	2
4	67.0	26
<i>N. gonorrhoeae</i>		
F62 (1)	5	—
" (2)	0	—

* Phagocytic activity observed in the urethral exudate from patients with gonococcal urethritis.

DESCRIPTION: *Neisseria gonorrhoeae* F62 Type 1 and pooled gibbon leukocytes were used throughout this study. The *in vitro* phagocytic system of Smith and Wood was employed.

Gonococcal suspension: F62 Type 1 gonococci were grown on GCBID agar for 15–16 hrs at 39°C in candle jars. Bacterial growth was scraped with a glass rod, washed off the plate with warm (39°C) GCBID broth and centrifuged at $1,400 \times g$ at 26°C for 20 min. The gonococci were resuspended in warm GCBID broth. The suspension was further agitated in a mixer for 2 min and diluted to a final concentration of 5×10^{10} gonococci per ml. The concentration of gonococci was determined by direct count in a Petroff–Hausser Chamber (C.A. Hausser and Son, Pa.). The viability of gonococci was evaluated by enumerating colony forming units on GCBID agar.

Gibbon leukocytes: Heparinized samples of gibbon whole blood were mixed with an equal volume of 6% dextran solution and centrifuged at $1,400 \times g$, at 4°C for 30 min. The buffy coat was harvested, resuspended in Hank's balanced salt solution supplemented with 0.1% glucose (HBG), and recentrifuged as previously described. The buffy coat of the second centrifugation now contained only a small number of red blood cells. Concentration of the leukocytes was determined by counting in a hemocytometer.

Phagocytosis: 1.25×10^9 gonococci, in a volume of 0.025 ml and 0.1 ml of HBG, were mixed with 2.5×10^8 packed gibbon leukocytes, and 0.06 ml of the mixture was distributed evenly over a 2×2 cm area of a glass slide. After incubation in a damp filter paper-lined petri dish chamber at 39°C for 30 min, the cells were recovered from the slide with 5 ml HBG and collected after centrifugation at $180 \times g$ for 3 min at 4°C. Smears were prepared and stained with methylene blue. The amount of phagocytosis was expressed as the per cent of 400 polymorphonuclear leukocytes (PMN) that contained one or more gonococci.

For determination of bactericidal activity, incubation of the phagocytic mixture was prolonged. Phagocytic mixtures were recovered after incubation at 39 C for 0, 1, and 2 hrs and viability determined by the viable count method. All tests were done in duplicate and the mean was taken as the result.

RESULTS: Table 3 presents the results of two identical experiments. Throughout this study, cultivation of gonococci and incubations of phagocytic mixtures were carried out at 39°C which is the average body temperature of gibbons.

Table 3. *In vitro* Phagocytic Activity of Gibbon Leukocytes and Viability of F62 Type 1 Gonococci in the Concentrated Test System after Incubation at 39° C.

Experiment No.	Phagocytosis 30 min (%)	Viability after 1 hr (%)	Viability after 2 hrs (%)
1	49	52	50
2	53	42	18
Average	51	47	34

DISCUSSION: High concentrations of gibbon leukocytes phagocytize gonococci efficiently at 39°C, presumably their optimal temperature; they also kill the virulent gonococci intracellularly. Further experiments will be carried out.

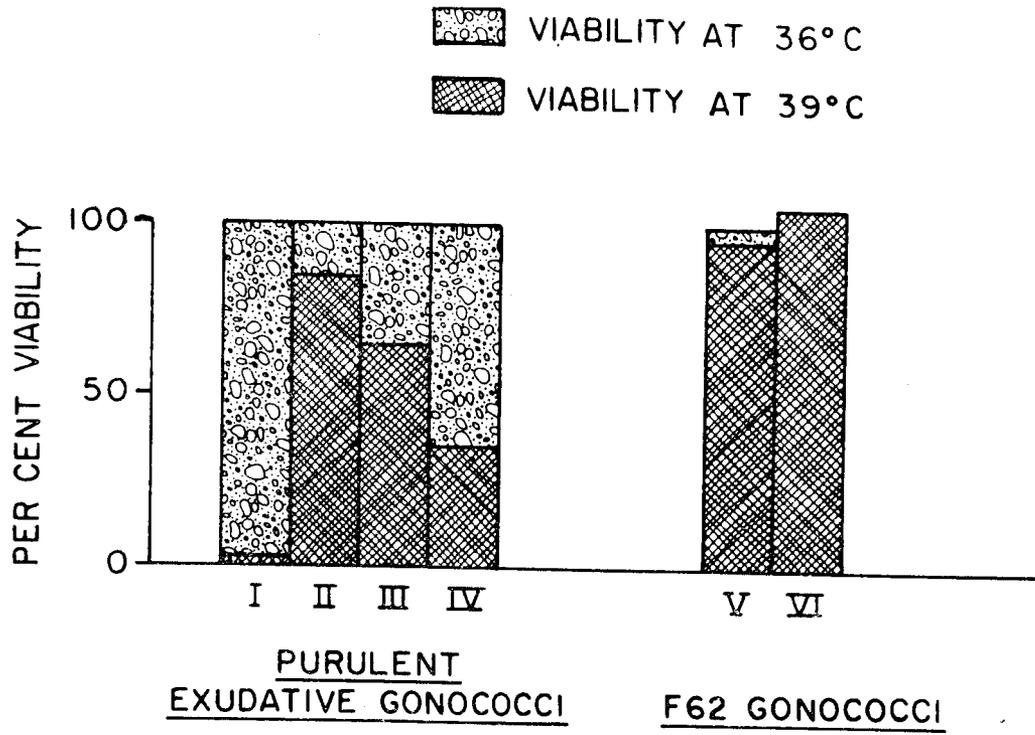


FIGURE I. VIABILITY OF TYPE I GONOCOCCI AT 36°C () AND AT 39°C () WHICH WAS EXPRESSED AS PER CENT OF VIABILITY AT 36°C. FOUR PURULENT URETHRAL EXUDATES (I-IV) WERE OBTAINED FROM FOUR MALE PATIENTS WITH ACUTE URETHRITIS. N. GONORRHOEAE F62 TYPE I WAS TESTED IN TWO EXPERIMENTS (V-VI). INCUBATION WAS TERMINATED AFTER 48 HRS.

PART IV: *Inoculation of Gibbons with an Exudative Gonococcal Suspension.*

DESCRIPTION: Four adult male gibbons were anesthetized and inoculated intra-urethrally via polyethylene catheters with 0.1 ml of gonococcal suspension prepared from pooled urethral discharges of 10 male patients with acute gonococcal urethritis and collected in a centrifuge tube containing 0.5 ml GCBID broth. The number of viable gonococcal colony forming units in each inoculum was determined. The gibbons were observed for any indication of infection, and bacteriological cultures, serum samples, and clinical observations were followed both prior to and after the inoculation.

RESULTS: Each gibbon was inoculated intra-urethrally with 5×10^6 viable gonococcal colony forming units. Bacteriological cultures showed little change in the microflora of the urethra, the conjunctival sac, or the rectum of the inoculated gibbons. Gonococci were absent in all cultures throughout 30 days of observation. During the course of observation, smears prepared from urethral exudates of the gibbons were uniformly negative for gram-negative diplococci, except in one instance where "one extracellular gram-negative diplococcus suspected" was reported in one gibbon on day 4 after inoculation. While bacteriological cultures of urethral exudate of this gibbon did not confirm the presence of gonococci, clinical observation was encouraging, i.e., the penis was reportedly reddened from day 5 to 11 and the normally clear exudate became purulent on day 13 through day 30. Three of the four gibbons developed some degree of inflammatory response as indicated by elevation of WBC, and by clear discharges. Results of the serological studies are not yet available.

DISCUSSION: The uniform absence of gonococci in cultures taken at the site of inoculation indicate failure of the dose of 5 million viable gonococcal colony forming units to establish infection in the urethra of the gibbon. Attempts to produce the infection with a larger inoculum of gonococci are underway.

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