INFECTION AND IMMUNITY, July 1973, p. 98-104

Vol. 8, No. 1
Printed in U.S.A.

Interaction of Gonococci with Phagocytic Leukocytes from Men and Mice

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Received for publication 13 March 1973

The interaction of human and mouse phagocytic leukocytes with representative virulent (F62-T1) and avirulent (F62-T4, RD-5) strains of *Neisseria gonorrhoeae* was studied in vitro. Leukocyte monolayers were incubated with gonococci for 30 min at 37 C, washed repeatedly, reincubated with fresh medium, and sampled for viable bacteria at intervals. After the initial incubation period and washing, human leukocytes retained larger numbers of viable T1 than of T4. During the subsequent 120 min of incubation, the numbers of viable T1 remained approximately constant, whereas viable counts of T4 declined by about two-thirds. In contrast, mouse leukocytes under similar conditions destroyed 70% of both types of gonococci. When human bactericidal serum was applied to infected human leukocytes, it had no effect on T4 but inactivated over 50% of T1. It is concluded that T4 are phagocytized by human leukocytes and are thus exposed to internal digestion, but are protected from bactericidal serum. T1, on the other hand, either adhere to the surface of the leukocytes or remain located so that they are neither digested by the leukocytes nor protected from bactericidal serum.

Man appears to be the only natural host for gonococcal infections, and attempts to infect other animals with *Neisseria gonorrhoeae* have been unsuccessful (6), except with the chimpanzee (11). Both serum and cellular defense mechanisms have been implicated in the host resistance mechanisms, but the results on which these conclusions are based are somewhat conflicting.

A report by Abdoosh (1) in 1936 suggested that man's susceptibility to gonococcal infections is due to a deficiency in normal serum bactericidins, which resistant hosts possess. More recent reports (22, 24), however, indicate that normal human serum is definitely bactericidal for many gonococcal strains in vitro. Although serum bactericidal factors may not be always operative in vivo, it would seem that gonococcal pathogenicity may involve factors other than ability to survive in human serum.

One such factor may be the survival of gonococci within human phagocytic leukocytes (2, 10, 12, 15, 17), in contrast to digestion within leukocytes of resistant hosts (23). This hypothesis is due, in part, to the frequent observation that gonococci are associated with polymorphonuclear leukocytes in urethral exudate material from patients with acute gonococcal urethritis (13, 20) and in the spinal fluid from a case of gonococcal meningitis (19).

The description of virulent and avirulent gonococcal colonial types by Kellogg et al. (9. 10) provided a new approach to the study of gonococcal pathogenesis. Organisms from infections freshly isolated on laboratory culture media formed characteristic colonies designated as type 1 (T1). Other distinct colonial types (T3 and T4) rapidly appeared upon nonselective subculture and eventually comprised the major types. Kellogg et al. (10) selectively transferred a T1 and its derivative T4 type for 69 consecutive passages and found that the T1 was still capable of producing acute urethritis in male volunteers, whereas the T4 was not. This study provided a basis for ascribing virulent properties to the colonial type T1 and avirulent properties to type T4.

It thus appears that the mechanisms of gonococcal pathogenicity may involve both the pathogen and host. We studied the interaction of virulent and avirulent gonococci with phagocytic leukocytes from both a susceptible and a resistant host. We adapted to our use the technique of Craig and Suter (4) and of Watt (23) who used leukocytes adhering to glass, which can be washed free of bacteria remaining in the supernatant fluid. We paid particular attention to factors that influenced the interaction between host and pathogen after initial association had been established.

MATERIALS AND METHODS

Bacterial cultures. *N. gonorrhoeae* strains F62-T1 and RD-5 were obtained from Douglas Kellogg, Center for Disease Control, Atlanta, Ga. A T4 colony type was selected from the F62-T1 culture and selectively passaged eight times on GC medium base (Difco) supplemented with Isovitalex (GCI; BBL), or with supplements I and II (GCS; 16). These media were used interchangeably during this investigation with no detectable alteration of the T1 and T4 colony types. Stable populations of F62-T1 and F62-T4 showed approximately 98% T1 and 100% T4 colony types, respectively. RD-5, a laboratory strain (10), was 100% T4 type. The bacteria were suspended in Tryptic soy broth (Difco) containing 20% glycerol (vol/vol), and were stored at −80 C in 1-ml samples. For each experiment, a vial was thawed, and 0.1-ml portions were spread on the surface of GCI plates and incubated for 16 to 18 h in 7% CO_2 at 37 C. The organisms were harvested with medium 199 containing 25 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) buffer (M199H; Grand Island Biological Co.), pH 7.2, and diluted to the desired concentration in the same medium containing 10% heat-inactivated human or fetal calf plasma. Alternatively, biphasic GCI (16) was inoculated with the harvest from agar plates, and gonococci in early logarithmic growth phase were harvested by centrifugation, washed once with M199H, and suspended as described above.

Human leukocytes and plasma. Human blood (15 to 20 ml) was collected by venipuncture into plastic syringes containing heparin (approximately 200 units; Grand Island Biological Co.). The syringe was placed in an upright position for 90 min at room temperature to allow for erythrocyte sedimentation. The leukocyte-rich plasma layer was collected in sterile siliconized glass tubes and centrifuged at 250 × *g* for 15 min at 20 C. The plasma was decanted and treated separately. The cells were suspended in phosphate-buffered saline containing 100 units of heparin per ml, and the cell suspension was centrifuged at 164 × *g* for 30 min at 20 C. The phosphate-buffered saline was discarded, and the leukocytes were suspended in M199H plus 10% heat-inactivated plasma, prepared as follows. The initial decanted plasma portion was heated at 56 C for 30 min and centrifuged at 237 × *g* for 5 min. The supernatant fluid was diluted to 10% in M199H and filter-sterilized before use. Leukocyte preparations throughout this study were prepared primarily from one donor. In all cases, the leukocyte population was composed of approximately 70% polymorphonuclear leukocytes (PMN) and 30% mononuclear leukocytes (MN). Viability of the leukocytes determined by dye exclusion (0.15% eosin) ranged from 95 to 99%.

Mouse leukocytes. Male or female Swiss white mice (NMRI-NIH inbred strain), weighing 20 to 25 g, were injected intraperitoneally with 2.0 ml of sterile Tryptic soy broth to stimulate granulocyte infiltration. The mice were reinjected intraperitoneally 210 min later with 2.0 ml of M199H containing 10% heat-inactivated (56 C, 30 min) fetal bovine serum (HIFBS; Grand Island Biological Co.). Eight to ten mice were killed by cervical dislocation, and the peritoneal fluids containing the leukocytes were pooled in sterile, siliconized glass tubes. After removal of the fibrin clot, the leukocytes were examined and enumerated in a hemocytometer chamber. The leukocyte population contained approximately 70% PMN and 30% MN and were 95 to 99% viable as assayed by the dye exclusion technique.

Phagocytosis and killing experiments. The method used for gonococcal phagocytosis by human and mouse leukocytes was a modification of the technique of Craig and Suter (4). Human leukocyte suspensions (0.3 ml containing 10^7 to 2×10^7 leukocytes) were pipetted into dram vials containing glass cover slips (12 mm diameter). The vials were sealed with rubber stoppers and incubated at 37 C for 30 min to allow the phagocytes to attach to the cover slips. The leukocytes were then washed twice with 1.0-ml portions of M199 containing Hanks balanced salt solution or HEPES buffer. The leukocyte cultures were then inoculated with T1 or T4 gonococci suspended in M199H containing heat-inactivated plasma (0.2 ml containing 3×10^7 to 6×10^7 bacteria).

Mouse leukocyte cultures were prepared in dram vials, as above, with the following alterations in technique. After distribution to the vials (approximately 10^6 leukocytes/vial), the cultures were centrifuged at 27 × *g* for 10 min at 20 C to increase the contact of the cells with the glass cover slips. The cells were then allowed to attach to the glass by incubation at 37 C for 60 min and were washed once with 1.0 ml of M199H. The cultures were inoculated with T1 or T4 gonococci suspended in M199H containing HIFBS (0.5 ml containing 10^7 to 2×10^7 bacteria).

Both human and mouse leukocyte preparations were incubated with the gonococci at 37 C for 30 min and then washed to remove free bacteria. Human leukocyte cultures were rinsed five times, and mouse leukocyte cultures were washed twice with 1.0-ml amounts of M199 containing Hanks balanced salt solution or HEPES buffer. The final suspending medium for both cell species was 1.0 ml of M199H, incorporating in some experiments 10% unheated or heat-inactivated human or fetal bovine serum. The vials were then incubated at 37 C, and this was designated as zero time. At 0, 60, and 120 min of incubation, the vials were treated as follows. For enumeration of free bacteria, a sample of the medium was withdrawn and serially diluted in M199H, and duplicate 0.1-ml amounts of the appropriate dilutions were spread on the surface of GCS or GCI plates. Similar procedures were used to test the bacteria removed from the cultures and in the washes. Cell-associated gonococci were enumerated by removing the remaining medium and lysing the leukocytes by the addition of 1.0 ml of distilled water containing 0.01% bovine plasma albumin (Metrix) and by agitation on a Vortex mixer. The lysates were diluted and plated as described above. Killing of gonococci by the leukocytes was calculated from the initial count of cell-associated bacteria and the organisms recovered after various periods of incubation.

All inoculated GCI or GCS plates were incubated at 37 C for 16 to 20 h in the presence of 7% CO_2, and the colonies were counted with the aid of a dissecting

microscope. The results are expressed as colony-forming units (CFU) per culture.

Serum bactericidal assay. Human serum was obtained from five adult males with no history of gonococcal infection. All sera showed a negative indirect fluorescence test for gonococcal-specific antibody (H. A. Gaafar and D. D'Arcangelis, Abstr. Annu. Meet. Amer. Soc. Microbiol., p. 114, 1972; test results from W. D. Lawton, New York State Department of Health). Mouse serum was collected from the hearts of ten mice. Serum samples were stored at −80 C and used within 10 days of collection.

Growth from a 17-h GCI plate culture of gonococci was harvested in 2.0 ml of Dulbecco balanced salt solution (Grand Island Biological Co.) containing 0.1% gelatin (DSSG) and placed in tubes containing glass beads (type 106, 3M Co.). The tubes were agitated on a Vortex mixer to disperse bacterial clumps, and serial dilutions were made in DSSG to approximately 10^4 CFU/ml. Serum was diluted in DSSG and maintained at 4 C until used. The test mixture consisted of 0.1 ml of the gonococcal suspension added to 0.8 ml of DSSG and 0.1 ml of the appropriate serum dilution. Controls received 0.1 ml of heat-inactivated serum (56 C for 30 min). Tubes were incubated at 37 C in a shaking water bath for 30 min, and triplicate 0.1-ml amounts were withdrawn for plating on GCI or GCS. The CFU were enumerated at 20 to 24 h of incubation in the CO_2 incubator. Bactericidal titers were expressed as the reciprocal of the serum dilution killing 90% of the gonococci in comparison with the corresponding heat-inactivated serum controls.

RESULTS

Interaction of gonococci and human leukocytes. The result of the initial 30-min contact between the gonococci and the human leukocytes was evaluated by examining cover slips stained immediately after the five washes (zero time) and by viable counts of the original inocula, of organisms removed after the period of incubation and in the washes, and of the organisms remaining in the cultures. Most of the gonococci were seen, by microscopy, in association with leukocytes, and only an occasional bacterium appeared to be clearly separated from them. About 60 to 70% of the leukocytes were infected, with the majority containing one or few diplococci but rarely large numbers. Obvious differences between the appearance by light microscopy of T1- and T4-infected cells were not observed. Viable counts are presented in Table 1. Leukocyte cultures were infected with virtually the same numbers of F62-T1 or F62-T4 organisms (3×10^7 and 2.8×10^7, respectively), and about the same numbers of organisms were removed from these cultures (a total of about 85% of those added). There was a pronounced difference, however, in the numbers of T1 and T4 that remained associated with the leukocytes (2.6 and 0.21%, respectively). Table 1 does not provide a reliable measure of the inactivation of the bacteria, a fact attributable to their association with the leukocytes, but it does indicate that the viability loss was greater with T4 organisms.

The survival of the gonococci in the washed cultures during the next 2 h of incubation is illustrated in Tables 2 and 3. In the case of T4 (Table 2), at zero time about 10% of F62-T4 and 2.5% of RD-5 were in the medium, and the rest were cell associated. During subsequent intervals (60 and 120 min), the numbers of bacteria free in the medium remained approximately the same, whereas the number of bacteria that were cell associated declined. The decline of cell-associated RD-5 was more pronounced than was that of F62-T4, but the results with F62-T4 comparable with those of RD-5 were obtained with the cells of another donor (not shown). Similar killing patterns of avirulent gonococci were obtained with bacteria harvested after 16 to 18 h of growth on agar plates or from early logarithmic biphasic broth cultures. In contrast to T4, the number of viable F62-T1 in the culture medium (about 3% of the total), as well as those remaining in association with the leukocytes, remained about the same during the 2-h period of incubation (Table 3).

Effect of human serum on free and leukocyte-associated gonococci. The results of typical serum bactericidal tests are shown in Table 4. Normal unheated human serum incubated with gonococci for 30 min displayed considerable bactericidal activity in comparison with heated serum, and the titers were the same with F62-T1 and F62-T4 cells. In addition, one human serum proved to be bactericidal for 8 of 11 strains of *N. gonorrhoeae* tested (not shown). Both resistant strains were of the T1 and T4 colonial types. Normal mouse serum was not bactericidal for any of the strains or types in our collection, whereas normal rabbit serum inactivated all strains tested.

The effect of normal unheated human serum on F62-T4 and F62-T1 incubated for 30 min with human leukocytes is illustrated in Tables 5 and 6. These results were compared with the values obtained with heated serum at 30 min. Viability of both types of gonococci free in the medium was reduced to 2 to 4% by 30 min of incubation with normal unheated human serum. The effect on the cell-associated gonococci, however, was different for the two types. T4 was unaffected, whereas the viability of T1 was reduced to 33% (Table 5). The gonococci used in the experiment described above were obtained immediately after washing of the cul-

TABLE 1. *Viable counts of bacteria added, removed, and retained by leukocyte cultures after the initial 30 min of incubation at 37 C*

Time of bacteria sampling	F62-T1		F62-T4	
	Viable count[a]	%	Viable count[a]	%
After addition to leukocytes	3.0×10^7	(100)	2.8×10^7	(100)
After removal from leukocytes				
Original inoculum	2.4×10^7	80	2.3×10^7	82
Wash 1	6.8×10^5	2.3	8.2×10^5	2.9
Wash 2	3.8×10^5	1.3	1.0×10^5	0.36
Wash 3	0.85×10^5	0.28	0.35×10^5	0.12
Wash 4	0.65×10^5	0.21	0.10×10^5	0.04
Wash 5	0.44×10^5	0.15	0.09×10^5	0.03
After leukocyte association	7.8×10^5	2.6	0.58×10^5	0.21

[a] Determined by appropriate dilution and duplicate plating. The values for bacteria removed from and retained by the leukocytes are the means of duplicate samples.

TABLE 2. *Survival of avirulent N. gonorrhoeae types in human leukocytes*

Incubation time[b] (min)	Gonococci (CFU/ml)[a]			
	Medium		Cell-associated	
	F62-T4	RD-5	F62-T4	RD-5
0	6.3×10^3	1.7×10^4	5.7×10^4	6.8×10^5
60	9.9×10^3	1.3×10^4	1.5×10^4	1.2×10^5
120	6.2×10^3	1.1×10^4	1.8×10^4	7.3×10^4

[a] Each value represents the average of three samples. Appropriately diluted samples from each reaction vial were plated in duplicate. Data for F62-T4 and RD-5 were obtained from separate experiments. Values similar to those shown for F62-T4 were obtained in two additional experiments performed in triplicate.

[b] Time elapsed after allowing for initial association and washing procedures as described in Materials and Methods.

TABLE 3. *Survival of virulent N. gonorrhoeae F62-T1 in human leukocytes*

Incubation time[a] (min)	Gonococci (CFU/ml)[b]	
	Medium	Cell-associated
0	5.0×10^3	1.6×10^5
60	6.0×10^3	1.6×10^5
120	5.3×10^3	1.9×10^5

[a] Time elapsed after allowing for initial association and washing procedures.

[b] Each value represents the average of three samples. Appropriately diluted samples from each reaction vial were plated in duplicate. Similar values were obtained in two additional experiments performed in triplicate.

TABLE 4. *Bactericidal activity of normal human and mouse serum against F62-T1 and F62-T4 gonococci*

Serum source	Bactericidal titer[a]	
	F62-T1	F62-T4
JH (individual human)	80	80
FT (individual human)	160	160
Pooled human serum[b]	160	160
Pooled mouse serum[c]	<10	<10

[a] Reciprocal of the serum dilution killing at least 90% of the gonococci when compared with heat-inactivated serum controls. The viability of the gonococci was maintained somewhat better in heat-inactivated serum than in DSSG.

[b] From five individuals, including JH and FT.

[c] From ten mice.

tures. When the experiment was repeated with F62-T1 recovered from cultures incubated for 0, 30, or 90 min without serum, the results were not materially different (Table 6).

Interaction of gonococci and mouse leukocytes. The survival rates of T1 and T4 gonococci with mouse leukocytes are presented in Table 7. There was essentially no difference in the ability of mouse PMN to digest virulent or avirulent organisms. The differential survival of T1 gonococci, as previously observed with human phagocytes, was not demonstrable with mouse leukocytes.

DISCUSSION

The kinetics of ingestion and killing of certain bacteria by leukocytes can be best studied by gently tumbling the reactants in suspension cultures (3, 7, 14). In our hands, this technique was not satisfactory for gonococci because T1 organisms tended to aggregate, and both types were unstable in control tubes containing no leukocytes. We resorted, therefore, to a technique that permitted some observations on the initial period (Table 1) but illustrated predominantly the subsequent phase of leukocyte-gonococcus interactions.

TABLE 5. *Effects of normal and heat-inactivated human serum on leukocyte-associated N. gonorrhoeae F62-T4 and F62-T1*[a]

Gonococcal type	Serum	Gonococci (CFU/ml)	
		Medium	Cell-associated
T4	HI	1.9×10^3	4.8×10^3
	Normal	4.9×10^1	4.3×10^3
T1	HI	1.2×10^4	1.2×10^5
	Normal	4.5×10^2	4.0×10^4

[a] Leukocytes were infected as described in Materials and Methods. At zero time, the suspending medium was 1.0 ml of M199H containing 10% normal serum or 10% heat-inactivated (HI) serum. After 30 min of incubation at 37 C, the cultures were sampled. Each value represents the average of four cultures. The samples from the medium and lysed leukocytes of each culture were plated in duplicate. Four other experiments, one with cells of another donor, yielded similar results.

TABLE 6. *Effects of normal and heat-inactivated human serum at postinfection intervals on leukocyte-associated N. gonorrhoeae F62-T1*

Time of serum addition[a] (min)	Serum	Gonococci (CFU/ml)[b]	
		Medium	Cell-associated
0	Normal	2.9×10^3	1.6×10^5
	HI	4.7×10^4	8.4×10^5
30	Normal	2.4×10^3	1.5×10^5
	HI	3.0×10^4	5.2×10^5
90	Normal	3.2×10^3	3.4×10^5
	HI	5.6×10^4	7.7×10^5

[a] Leukocytes were infected with gonococci and incubated as described in Materials and Methods. At 0, 30, or 90 min after incubation, the culture medium was removed and replaced with 1.0 ml amounts of M199H containing 10% normal or heat-inactivated (HI; 56 C for 30 min) human serum. After 30 min of exposure to the sera, the contents of the vials were sampled as described in Materials and Methods.

[b] Each value represents the average of two cultures. Another experiment, also performed in duplicate, gave similar results.

The results described in this paper reveal subtle differences in the biological properties of F62-T4 and F62-T1 gonococci as reflected in their interactions with human leukocytes. Both types are highly susceptible to the action of normal serum (Table 4) and both become readily associated with human leukocytes, but the nature of this association differs. The viability of T4 organisms declines in leukocyte cultures, whereas the viability of T1 bacteria remains approximately constant (Tables 1-3). A simplistic explanation of these results is that both types are phagocytized but T4 is digested, whereas T1, by some unknown mechanism, resists digestion. The results shown in Table 5 cast some doubt on the intracellular location of T1. Normal unheated human serum has no effect on the viability of T4 associated with leukocytes but inactivates a large fraction of the cell-associated T1. It therefore appears that some of the T1 are intracellular, whereas the majority either adhere to the surface of the leukocytes or are located so that they can interact with extracellular serum. In either case, the proportion of serum-resistant and serum-susceptible organisms remains approximately the same during an incubation period of 90 min (Table 6). Inactivation of the gonococci by normal serum may reflect opsonin activity, but destruction in the absence of leukocytes is even more rapid, and the results shown most likely represent bactericidal activity (Tables 5 and 6). It therefore appears that the interaction between T1 and T4 with human leukocytes involves factors that have contrasting effects. T4 is phagocytized, but while it is being digested at a moderate rate it is not exposed to bactericidal serum. T1 is not digested by the leukocytes, but it is not internalized sufficiently well to be protected from extracellular serum.

It is not known to what extent the observations described above reflect the relationship of naturally occurring strains of the gonococcus to human leukocytes, but a few comparisons can be made with the results of other investigators. Watt (23), by using three fresh gonococcal isolates, showed that 80 to 85% of the bacteria were killed by human leukocytes during the first hour, but there was virtually no further killing during the following 2 h. Since his work was done with the leukocytes from a single donor, it

TABLE 7. *Survival of virulent and avirulent N. gonorrhoeae types in mouse leukocytes*

Incubation time[b] (min)	Gonococci (CFU/ml)[a]			
	Medium		Cell-associated	
	F62-T4	F62-T1	F62-T4	F62-T1
0	8.3×10^3	4.1×10^3	2.9×10^4	5.1×10^4
60	5.4×10^3	1.3×10^3	1.2×10^4	1.7×10^4
120	5.0×10^3	1.2×10^3	8.5×10^3	9.4×10^3

[a] Each value represents the average of two samples. Appropriately diluted samples from each reaction vial were plated in duplicate. Similar results were obtained in two other experiments performed in duplicate.

[b] Time elapsed after allowing for initial association and washing procedures as described in Materials and Methods.

is not known if the difference during the first hour of incubation between his results and ours was due to the source of leukocytes or to the bacterial strains. As in our case, however, Watt (23) came to the conclusion that the surviving gonococci were not necessarily phagocytized, because they remained susceptible to the action of penicillin. We obtained evidence of a similar nature by the use of normal human serum. Watt (23) also showed that the leukocytes of the guinea pig (a resistant host) rapidly killed the gonococci, as was the case in our experiments with mouse leukocytes (Table 7).

There is considerable evidence that the bactericidal activity of normal human serum against gonococci is the result of a complement-mediated antibody-antigen reaction (5). Since the bactericidal activity of normal human serum can be demonstrated with numerous gonococcal strains (8 of 11 in our case) of T1 and T4 colony types, it is difficult to explain the survival advantage in man of T1 over T4. Ward et al. (22) have shown, however, that gonococci in urethral exudates are resistant to serum bactericidal activity but become sensitive when cultured in vitro. Conversely, Watt et al. (24) have shown that phenotypically virulent colony types, initially serum sensitive, become resistant when cultured on a medium containing prostatic extract. It is possible, therefore, that gonococcal virulence is ultimately determined by characteristics reflected in colonial morphology which prevent phagocytosis and digestion and additional alterations allowing for resistance to serum bactericidal activity.

Because virulent gonococci possess pili (8, 18), Swanson et al. (18) suggested that piliation may hinder phagocytosis in a manner analogous to encapsulated bacteria. In support of this hypothesis, it has been reported that T4 gonococci, which lack pili, are phagocytized more readily than are T1 (C. G. Thonghai and W. D. Sawyer, Bacteriol. Proc., p. 110, 1971). An alternate hypothesis (18) is that pili promote adherence to host epithelial cells and allow for early colonization. It is possible, in view of these hypotheses, that T1 organisms adhere to the surface of leukocytes due to their sticky nature, but total engulfment does not occur because the overall surface tension of the organisms is less than that of the leukocyte. Van Oss and Gillman (21) demonstrated that bacteria with a surface tension less than that of the leukocyte are not phagocytized as readily as are bacteria with a higher surface tension. Their conclusion that gonococci should be phagocytized readily may refer to the T4 colony type. In investigations of the factors responsible for the virulence of the gonococcus, it is particularly useful to use T1 and T4 colony types derived from the same isolate.

In conclusion, our results are in agreement with those of Watt (23) and further clarify one aspect of the different relationships of virulent and avirulent gonococci to human leukocytes.

ACKNOWLEDGMENTS

The authors express their sincere appreciation to Emilio Weiss of this laboratory for his interest and criticisms during the course of this investigation and in preparation of the manuscript. We are indebted to John E. Nutter, Armed Forces Radiobiology Research Institute, Bethesda, Md., for his many stimulating and helpful discussions. Technical assistance from Bonnie L. Horn is gratefully acknowledged.

This investigation was supported by the Bureau of Medicine and Surgery, Department of the Navy, research task MRO 41.09.01-012B6GJ.

LITERATURE CITED

1. Abdoosh, Y. B. 1936. Natural and immune bactericidins for the *Gonococcus*. J. Hygiene **36**:355-362.
2. Carpenter, P. L. 1965. Immunology and serology, 2nd ed., p. 308. W. B. Saunders Co., Philadelphia.
3. Cohn, A. Z., and S. I. Morse. 1959. Interactions between rabbit polymorphonuclear leukocytes and staphylococci. J. Exp. Med. **100**:419-443.
4. Craig, C. P., and E. Suter. 1966. Extracellular factors influencing staphylocidal capacity of human polymorphonuclear leukocytes. J. Immunol. **97**:287-296.
5. Glynn, A. A., and M. E. Ward. 1970. Nature and heterogeneity of the antigens of *Neisseria gonorrhoeae* involved in the serum bactericidal reaction. Infect. Immunity **2**:162-168.
6. Hill, J. H. 1944. Experimental infection with *Neisseria gonorrhoeae*. II. Animal inoculation. Amer. J. Syph. Gonor. Vener. Dis. **28**:334-378, 470-510.
7. Hirsch, J. G., and B. Strauss. 1964. Studies on heat-labile opsonin in rabbit serum. J. Immunol. **29**:145-154.
8. Jephcott, A. E., A. Reyn, and A. Birch-Anderson. 1971. *Neisseria gonorrhoeae*. III. Demonstration of presumed appendages to cells from different colony types. Acta Pathol. Microbiol. Scand., Section B. **79**:437-439.
9. Kellogg, D. S., I. R. Cohen, L. C. Norins, A. L. Schroeter, and G. Reising. 1968. *Neisseria gonorrhoeae*. II. Colonial variation and pathogenicity during 35 months in vitro. J. Bacteriol. **96**:596-605.
10. Kellogg, D. S., W. L. Peacock, W. E. Deacon, L. Brown, and C. I. Pirkle. 1963. *Neisseria gonorrhoeae*. I. Virulence genetically linked to clonal variation. J. Bacteriol. **85**:1274-1279.
11. Lucas, C. T., F. Chandler, J. E. Martin, and J. D. Schmale. 1971. Transfer of gonococcal urethritis from man to chimpanzee. J. Amer. Med. Ass. **216**:1612-1614.
12. Ovcinnikov, N. M., A. A. Bohenek, and P. B. Litvak. 1934. Phagocytosis or endocytobiotics, its significance in *Gonorrhoeae*. Urologiya **11**:84-89.
13. Ovcinnikov, N. M., and V. V. Delektorskij. 1971. Electron microscope studies of gonococci in the urethral secretions of patients with gonorrhoea. Brit. J. Vener. Dis. **47**:419-438.
14. Roberts, R. B. 1967. The interaction in vitro between group B meningococci and rabbit polymorphonuclear leukocytes. J. Exp. Med. **126**:795-817.
15. Smith, H. 1968. Biochemical challenge of microbial pathogenicity. Bacteriol. Rev. **32**:164-186.
16. Sparling, P. F. 1966. Genetic transformation of *Neisseria*

gonorrhoeae to streptomycin resistance. J. Bacteriol. **92**:1364-1371.
17. Spink, W. W., and C. S. Keefer. 1937. Studies of gonococcal infection. I. A study of the mode of destruction of the gonococcus *in vitro*. J. Clin. Invest. **16**:169-176.
18. Swanson, J., S. J. Kraus, and E. C. Gotschlich. 1971. Studies on gonococcus infection. I. Pili and zones of adhesion: their relation to gonococcal growth patterns. J. Exp. Med. **134**:886-906.
19. Swierczewski, J. A., E. J. Mason, P. B. Cabrena, and M. Liber. 1970. Fulminating meningitis with Waterhouse-Friderichsen syndrome due to *Neisseria gonorrhoeae*. Amer. J. Clin. Pathol. **54**:202-204.
20. Thayer, J. D., and W. Garson. 1965. The gonococcus, p. 451-468. *In* R. J. Dubos and J. G. Hirsch (ed.), Bacterial and mycotic infections of man, 4th ed. J. B. Lippincott Co., Philadelphia.
21. Van Oss, C. J., and C. F. Gillman. 1972. Phagocytosis as a surface phenomenon. 1. Contact angles and phagocytosis of non-opsonized bacteria. Res. J. Reticuloendothel. Soc. **12**:283-292.
22. Ward, M. E., P. J. Watt, and A. A. Glynn. 1970. Gonococci in urethral exudates possess a virulence factor lost on subculture. Nature (London) **227**:382-384.
23. Watt, P. J. 1970. The fate of gonococci in polymorphonuclear leucocytes. J. Med. Microbiol. **3**:501-509.
24. Watt, P. J., A. A. Glynn, and M. E. Ward. 1972. Maintenance of virulent gonococci in laboratory culture. Nature N. Biol. **236**:186.