

## *Neisseria gonorrhoeae*

### II. Colonial Variation and Pathogenicity During 35 Months In Vitro

DOUGLAS S. KELLOGG, JR., IRUN R. COHEN, LESLIE C. NORINS,  
ARNOLD L. SCHROETER, AND GILBERT REISING

*Venereal Disease Research Laboratory, National Communicable Disease Center, Atlanta, Georgia 30333*

Received for publication 15 June 1968

During 35 months of selective in vitro cultivation, *Neisseria gonorrhoeae* cells retained their virulence for humans and were shown to be closely related to a particular colonial morphology. Saline-autoagglutinability was the only other characteristic distinguishing virulent from avirulent cells. Human responses to challenge with cells of the different colonial types were studied for their relationships to virulence or avirulence.

Since its initial cultivation in 1882, *Neisseria gonorrhoeae* has been extensively studied as an academic challenge and as a practical medical diagnostic problem. In spite of such study, little had been ascertained about the immunological response of humans to a gonococcal infection. Immunity appeared to be transient or limited in nature as evidenced by repeated reinfections and the development of chronic conditions and carrier states. Antibody production could be detected by complement-fixation procedures using extracted antigens; however, there was not a close correlation of serological reactivity with infection (13, 19). Studies of the antigenic mosaic of *N. gonorrhoeae* demonstrated antigenic variation among strains and considerable sharing of antigens with other species of *Neisseria* as well as other genera of bacteria (24). Most studies of *N. gonorrhoeae* antigens were done with strains which had been carried in vitro long enough to obtain a sufficient number of cells for antigenic analysis. In vitro cultivation of *N. gonorrhoeae* resulted in a conversion of normal saline autoagglutinability from rough to smooth, possibly due to an antigenic alteration, and a loss of virulence. Since man was the only known host for *N. gonorrhoeae*, and usually was infected only by venereal contact, little was known about virulence of *N. gonorrhoeae* except for the possible involvement of a potent endotoxin which avirulent strains were known to possess. Progress in ascertaining basic facts about *N. gonorrhoeae* was hampered by a double deficiency: lack of an experimental animal and lack of a marker associated with virulence. In 1963, four clonal types were described for *N. gonorrhoeae* and a correlation was demonstrated between colonial morphology and virulence with two of the four colonial types (12). At that time

(after 69 selective transfers in vitro), cells of colonial type T1 were found to be virulent and cells of colonial type T4 were found to be avirulent for human volunteers. Virulence testing could not be attempted with cells of either colonial type T2 or T3 after 69 selective transfers in vitro because of time and space limitations. At 38 transfers, infections resulted from inoculations of cells of colonial types T1, T2, T3, and T4; however, the appearance of signs and symptoms was paralleled by an alteration in recovered colony types from T2, T3, or T4 to T1. At that point, T1 colonies could still be isolated from cultures of colonial types T2, T3, and T4. This paper presents the results of further studies of virulence of *N. gonorrhoeae* for humans, and certain characteristics of the infectious process and the organisms themselves which may be related to their virulence and overall interaction with the host.

#### MATERIALS AND METHODS

*N. gonorrhoeae* strain F62 was originally isolated by this laboratory in 1962 (12). Primary isolates were obtained at the Fulton County Health Department, Atlanta, Ga., through the assistance and cooperation of John H. Tiedemann. All colonial lines of strains were passaged by loop transfer of individual colonies selected by morphological characteristics observed by means of an AO Cycloptic dissecting microscope with diffused, angled light transmitted from below up through the medium. Measurements of colonial diameter during iron studies were accomplished with 20-fold magnification and an ocular micrometer. On three separate occasions, approximately 100 well-isolated colonies were measured at each compound level under study. All transfers were made after 16 to 20 hr of incubation at 35 C under increased carbon dioxide tension (candle extinction). G C Medium Base (GCB; Difco), enriched with a defined supplement (DSF), was used for the isolation and cultivation of

all strains (22). The defined supplement was added to the basal medium at 43 to 45 C just before pouring. When hemolyzed whole rabbit blood (5%) supplement was used, it was added with the defined supplement. In examinations of pharyngeal and rectal specimens, a selective medium was used (20). The fermentation medium utilized for confirmation of species identity was described by White and Kellogg (23). The synthetic medium used was described by Gould, Kane, and Mueller (8). Demonstration of capsules was attempted by a technique in which suspensions of *N. gonorrhoeae* in 10% sterile skim milk are examined with fixed-phase optics (6). The fluorescent-antibody (FA) procedures applied to direct smears, and/or smears of cultures, were described by White and Kellogg (22). The precipitin procedure for serum antibodies and control sera production was that described by Reising and Kellogg (16). The oxidase procedure was performed as described in U.S. Public Health Service Publication 499 (21). Assays for the presence of hemolysin, coagulase, and fibrinolysin were conducted according to standard procedures described for other microorganisms (9). Toxic potential of the cell types was assayed by intraperitoneal injection of equivalent numbers of each cell type into CFW mice. Antibiotic susceptibilities of the strains used for volunteer inoculation were determined by James D. Thayer of the Venereal Disease Research Laboratory.

After 17 months of passage, the four colony types of *N. gonorrhoeae* (T1, T2, T3, and T4) were tested for virulence with separate groups of four subjects. After 35 months of passage, only the colony type T1 was tested for virulence and a group of 10 subjects was used.

All subjects were male volunteers at Atlanta, Ga. Before examination as a possible subject for the study, each volunteer provided assurance that he understood the purpose of the experimentation and the possible risks involved. Selection of the subjects for the study required satisfaction of the following criteria. He must have been between ages 21 and 45 years and available for a poststudy observation period of 6 months. He must have been acceptable from the standpoint of mentality, psyche, and ability to cooperate. To be acceptable, the subject must not have had a gonococcal infection within the previous 3 months nor have been an asymptomatic carrier of *N. gonorrhoeae*; neither could he harbor an acute or chronic disease or psychogenic disorder. Freedom from gonococcal infection or carrier state was determined by clinical and laboratory examinations of urethra, prostate, rectum, and pharynx. A base-line laboratory examination included white blood-cell count, differential count, and hemoglobin, as well as a Venereal Disease Research Laboratory test and routine urinalysis.

Procedurally, each selected subject was placed in a restricted ward area the day before inoculation. On inoculation day, a set of GCBDSF agar plates with a 16- to 18-hr growth of the gonococcal strain to be used for inoculation was examined for the purity of colonial type. Where individual colonies or areas of colonies of other than the desired type were observed, the growth was marked with a dye. Immediately before the subject was inoculated, a sample of blood and a

loop sample of the urethral canal surfaces were obtained. Each subject received a full 2-mm bacteriological loop of the desired colonial type of cells picked only from areas free of contaminating colonial types. Each inoculum was inserted approximately 2 to 3 inches (5 to 7.6 cm) into the urethra, and the subject was instructed not to urinate for several hours. Daily samples for examinations were taken by inserting the loop into the urethra. Each daily specimen from each subject was examined by FA techniques and cultural procedures (colony morphology, oxidase reactivity, sugar fermentations, and Gram reaction) to identify the infecting organism as *N. gonorrhoeae*. After termination of the infection study, each subject was adequately treated with either aqueous procaine penicillin G or oxytetracycline. The subject was examined 24, 48, and 72 hr after treatment for signs of infection, and the urethra, urine, and prostate were sampled for the presence of *N. gonorrhoeae* by FA and cultural procedures. Volunteers were not released from the study ward until three successive daily examinations were negative for *N. gonorrhoeae* by all procedures. Blood specimens for serological examination were collected at intervals over the next 3 months.

## RESULTS

*Colonial morphology in N. gonorrhoeae.* The four colonial types originally described have been found to represent the most stable morphological configurations for the conditions of their cultivation. There are a variety of temporary morphological variations which appear as a result of environmental alterations, as well as some variations which are capable of perpetuating themselves under the conditions of selective transfer. As a consequence, in the selective transfer of the colonial types, we have adhered as closely as possible to the originally described characteristics in picking colonies for transfer. The original strain (F62) colonial types, which have been carried as separate entities for 3.5 years, are morphologically very similar to colonial types obtained from samples that were frozen or lyophilized in 1963. The two colonial types, T1 and T2, obtained from patients are characterized by their glistening convexity, dark-brown to black coloration, and small size (0.5 mm and 0.4 mm, respectively; Fig. 1). These characteristics are apparent only when observed on a transparent medium with the use of diffuse, angled light transmitted through the medium from below the plate. The colonial type T2 differs from T1 in having a slight internal granularity, a very marked definition of colonial edge, a friable consistency, and a greater ability to reflect light. The last characteristic, which is observed with a combination of transmitted and edge lighting, is consistent with T2 colonies having a thicker surface film of reflective material than T1 colonies. In Fig. 1, several points are of interest as they relate to colonial structure. Evidence of the

distinctions between T1 and T2 colonies can be seen in three aspects of the colonies: (i) the narrow crescent of refracted light (caused mainly by transmitted light from below the plate) at the upper edge of the T2 colony as compared with the broad crescent on the T1 colony; (ii) the bright highlight on the left side of the T2 colony (from edge lighting from second light source at side); and (iii) the sharply defined lower edge of the T2 colony. The T3 colony, large and granular, possesses an inner ring of lighter material which can be seen as a narrow light band running from left to right approximately halfway between the bottom edge and the center of the colony. Small irregular areas of granularity similar to that of the T3 colony can be seen in the colorless T4 colony. The lighting used in photographing these colonies was established in advance as optimal for depiction of colonial characteristics; then, without further adjustment, the individual colonies were photographed. Figure 2 illustrates the structural and

elevational characteristics of the four colonial types in a cross-section view.

Both T1 and T2 colonies were observed to acquire a surface roughness and a variation of colonial border which tends toward an intermediate colony type that we designated T21. Progeny of T21 colonies are predominantly colonial type T1, with the rest colonial type T2. The appearance of such colonies indicated some type of variation in the cultivation medium and served as an index to the reproducibility of our cultural conditions. Several hundred isolations of *N. gonorrhoeae* from both males and females further substantiated the relationship of these types with the disease state and the relative consistency of the morphological characteristics from strain to strain.

The two colonial types associated with nonselectively transferred laboratory strains of *N. gonorrhoeae* T3 and T4 are characterized by their nearly flat elevation, minimal coloration, and

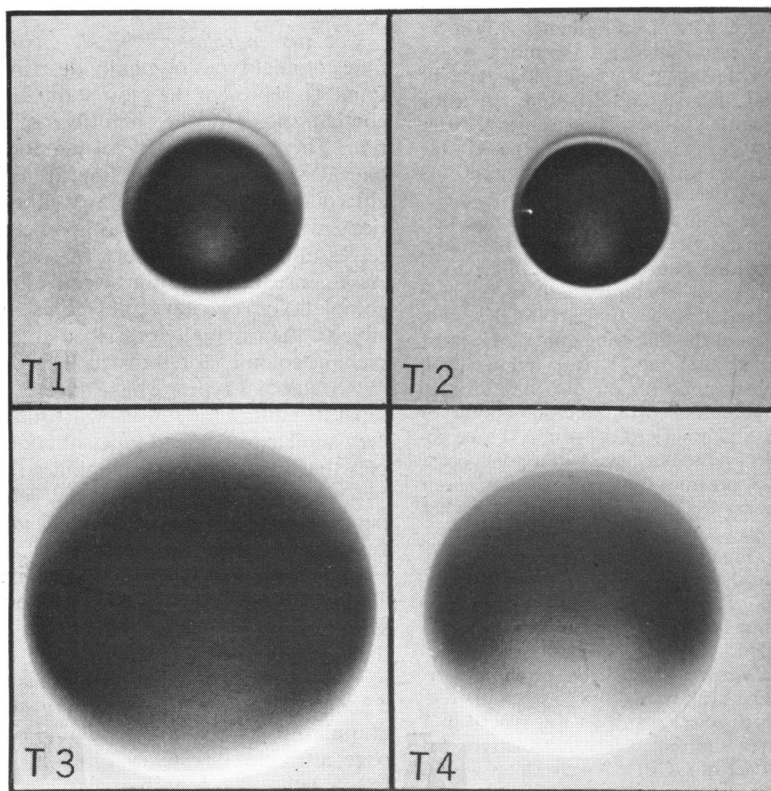


FIG. 1. Colonial types of *N. gonorrhoeae*. Combined illumination: transmitted light from below the plate with a ground-glass diffuser and tilted mirror, but no condensing system; edge lighting from second light source at side at  $15^\circ$  above flat plane.  $\times 58$ .

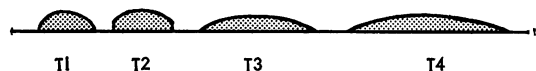


FIG. 2. Cross sections of colonies of *N. gonorrhoeae*.

larger size (1.0 to 1.2 mm). The T3 colonial type is distinguished from the T4 type by an internal light-brown granularity.

It must be emphasized that T1 and T2 colonial types can be maintained only by selective transfer of individual colonies having the typical morphology as observed through the dissecting microscope. All strains of *N. gonorrhoeae* studied under conditions of nonselective transfer demonstrate a conversion from colonial types T1 and/or T2 to colonial types T3 and/or T4. The conversion can be prevented only by selective transfer or storage. Unsuitable media complicated the maintenance of colonial types T1 and T2 by increasing the proportion of T3 and T4 colonial types found at each successive nonselective transfer. Stabilization of a strain as colonial type T1 or T2 has not been possible on any medium employed; however, with our particular medium, there was a selection of colonial type T1 for greater stability. For example, when colonial type T1 cells of a representative group of eight primary isolate strains were spread on agar-medium plates and the types of the resulting colonies were recorded, the results were as seen in Fig. 3. After four selective in vitro transfers of colonial type T1 cells, three strains were

well adapted and produced progeny, 98 to 99% of which were T1 colonies. Three strains produced progeny with only 50 to 65% T1 colonies; two strains produced 30% and 5% T1 colonies, respectively. Five selective passages later, all eight strains produced 98 to 99% colonial type T1 progeny. A more complex medium, such as hemolyzed whole rabbit blood-agar medium (5%), which supports excellent growth of *N. gonorrhoeae*, had no effect upon the assumption of stability, nor did a synthetic medium of simple composition. The synthetic medium, which supported sparse but equivalent growth in terms of numbers of clones of each of the four colonial types, did demonstrate a reduced rate of appearance of colonial types T3 and T4. This reduction could have been the result of the restricted amount of growth which allowed less opportunity for expressing a genetic change. Materials allowing colonial types T3 and T4 to make superior growth were absent, as indicated by the equivalent colony diameters of the four colonial types on the synthetic medium. All primary isolates grew on the synthetic medium.

After the first studies of colonial variation in *N. gonorrhoeae* (12), the apparent stepwise colonial step T1 to T4 selection in vitro could be readily reversed, another characteristic of subsequently isolated strains. However, after 15 months in vitro, strain F62 colonial type T4 was back-selected from T4 colonial morphology to T3 and then T2 morphology only. Colonial type T1

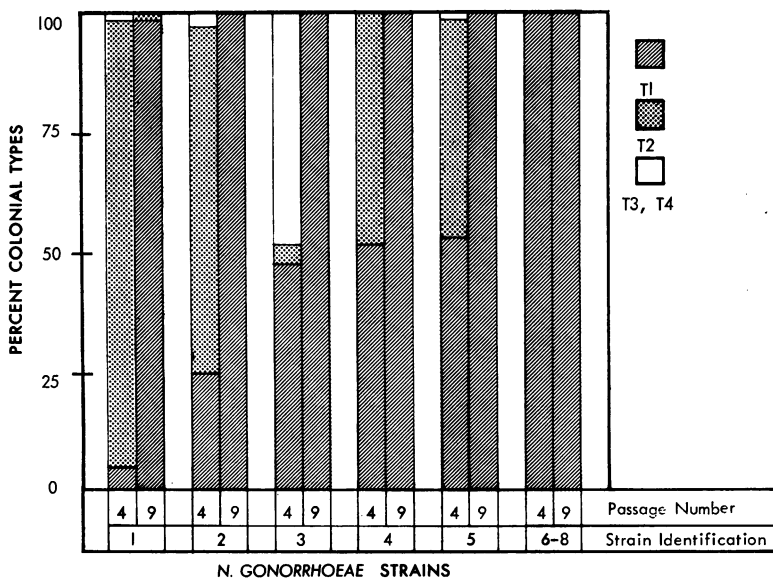


FIG. 3. Stabilization of colonial type in *N. gonorrhoeae* strains. Each passage in vitro was selective for T1 colonial morphology and was spread-inoculated for examination of percentage of individual colony types present.

clones were not detected in the back-selected T2 line, even though they were detected in the T2 line which had been carried as T2 for 15 months. Apparently, the T1 colonial characteristics were retained in the T2 inheritance with occasional expression, but were lost from the T4 inheritance after continued selection in vitro. Long term storage (1 month or longer) of the colonial types has been successful, either frozen in glycerol-broth at  $-40^{\circ}\text{C}$  or lyophilized. For routine management of active stocks for periods of less than one month or more than one weekend, we used 20% glycerol-G C Base Medium-broth and  $-20^{\circ}\text{C}$  storage. Some glycerol-broth ( $-20^{\circ}\text{C}$ ) stored suspensions were thawed, sampled, and re-frozen at least six times, and, although there was a decline in viable cells each time, there was no problem in obtaining adequate growth in 18 hr on agar-medium.

*Physiological characteristics of colonial types.*

The factors responsible for virulence are probably inherent in some aspect of the cellular physiology or antigenic mosaic which is reflected in the gross characteristic of colonial morphology. None of the characteristics previously associated with virulence in other microorganisms has been detected in *N. gonorrhoeae* cells. No evidence has been found for the presence of hemolysins, coagulases, or fibrinolysins. The toxic characteristics of the gonococcus are present in approximately equivalent concentrations in the cells of all four colonial types as assayed in CFW mice. No evidence of infection was observed in any of the mice during postmortem examinations. No distinct capsules were detected on any of the cells of the four colonial types, regardless of their source (primary isolates or laboratory strains) or cultivation medium. Cells of colonial type T2 exhibit a singular ability to grow throughout agar-medium shake tubes—a characteristic not shared by cells of the other three colonial types. The possible significance of this characteristic in terms of virulence is not known.

An outstanding requirement of both virulent and avirulent cells was for the presence of ferric ions in our medium. In Fig. 4, the logs of the concentrations of ferric ions (in micrograms per cent) are plotted against the relative increase in colonial diameters over the controls. Stimulation began at 5 to 10  $\mu\text{g}$  per cent and increased to a maximum at approximately 280  $\mu\text{g}$  per cent where the colonial size had doubled that of the controls. No further increase in colonial size was observed beyond 1,400  $\mu\text{g}$  per cent, and the only alteration of colonial morphology was an increase in the darkness of colonial coloration. The types of anions accompanying the ferric ions had no effect on the results.

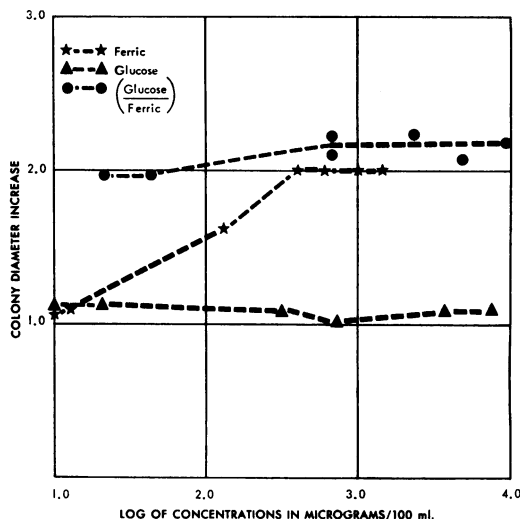


FIG. 4. Growth stimulation by iron ions. Growth on unsupplemented G C Base Medium equals 1.0. Glucose/ferric curve represents the effect of increasing glucose concentrations in the presence of a maximal stimulation level of ferric ions.

Glucose alone was ineffective, but with the ferric ions produced an additive effect. A ratio of glucose to ferric ions of less than one depressed the colony size to a level intermediate between that obtained with either additive alone. Hemin and ferrous gluconate were not stimulatory. Aluminum ions were nearly as effective as ferric ions, but manganese and magnesium ions were not stimulatory in our medium. The ferric ions could be incorporated into the medium before autoclaving without reducing the stimulation.

*Virulence studies with N. gonorrhoeae colonial types.* After 440 selective transfers in vitro over a period of 17 months, cells of strain F62 T1 and T2 colonies were found to be virulent for four male volunteers. Strain F62 T3 and T4 colonies were found avirulent at 17 months. There were several differences in the course of events in these men as compared to those of the previous study (12). Specimens were obtained from each man each day after infection and were tested by direct and delayed FA techniques (22) and cultural procedures.

Inoculation with cells of colonial types T3 and T4 resulted in either a watery discharge or none at all. Exudates and urethral scrapings from these volunteers contained rare FA-positive diplococci of poor morphology both intra- and extracellular to polymorphonuclear leukocytes at 24 hr postinoculation. Many polymorphonuclear leukocytes containing 2+ to 3+ FA-positive granules were seen, as well as occasional histiocytes and epithelial cells. No FA-positive diplococci were seen in

specimens taken at 48 hr postinoculation. The number of polymorphonuclear leukocytes in the exudates varied among the volunteers from few to many, and the FA-positive granules varied from 1+ to 3+ in intensity. Each 24-hr period postinoculation saw a decreasing number of polymorphonuclear leukocytes with decreasing FA-staining intensity of the granules in the exudates. The volunteers acquired a considerable tenderness of the inguinal lymph nodes starting about 24 to 36 hr postinoculation and lasting 3 to 4 days, with progressively decreasing severity. *N. gonorrhoeae* was not recovered in cultures from these volunteers at 24 hr postinoculation, or at any subsequent time.

Inoculation with cells of colonial types T1 or T2 resulted in the formation of moderate amounts of purulent exudate. These exudates contained many FA-positive diplococci intra- and extracellular to polymorphonuclear leukocytes. Histiocytes, epithelial cells, and many polymorphonuclear leukocytes, containing 1+ FA-positive granules, were observed. By 72 hr post inoculation, the proportions of intra- and extracellular FA-positive diplococci in relation to polymorphonuclear leukocytes had begun to shift to more extracellular than intracellular. Between 96 hr and 120 hr, the shift was nearly complete and only rare intracellular diplococci were seen. By 168 hr and 192 hr, there were varying numbers of extracellular diplococci among the volunteers, and many polymorphonuclear leukocytes which contained 3+

FA-positive granules. The extracellular diplococci had a poor morphological appearance which could be described as "moth eaten." The considerable tenderness of the inguinal lymph nodes seen in the volunteers who received colonial types T3 and T4 was not seen in the volunteers who received colonial types T1 and T2.

An interesting situation was observed when the daily isolates from the infected volunteers were examined for their relative percentages of T1 and T2 colonial types. Colonial types T3 and T4 were so rare that they were not included in the determinations. In Fig. 5, the percentages of type T1 and T2 colonies per isolate are shown for each day postinoculation. When the inoculum was 100% T1 cells, successive daily isolates showed a progressive change in the composition of the population to 90% T2 colonies. A similar change was seen when the inoculum was colonial type T2; i.e., altered to 90% colonial type T1. The number of colonies per sample was approximately the same for each volunteer, as was the exudate content (number of polymorphonuclear leukocytes, intra- and extracellular FA-positive diplococci, and tissue cells). The rate of colonial change was about the same among volunteers who received colonial type T1 cells. However, the rate of colonial change showed more variation with the individual men who had received colonial type T2 cells (Fig. 6). By the 2nd day, the colonial types from two of the volunteers had altered to greater than 70% colonial type T1. The exudate from one volunteer did

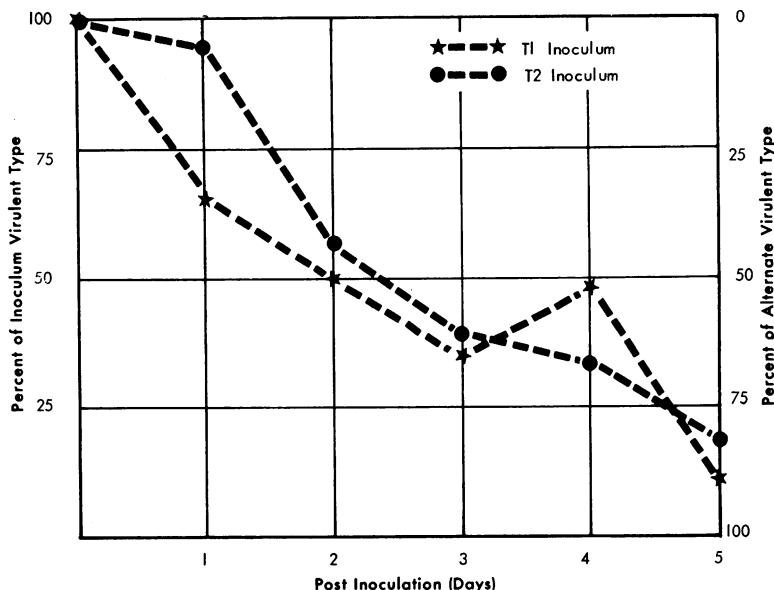


FIG. 5. Alteration of colony type in exudates. Average percentage of T1 and T2 colonies found on successive days after inoculation, considering total number of T1 and T2 colonies to equal 100%.

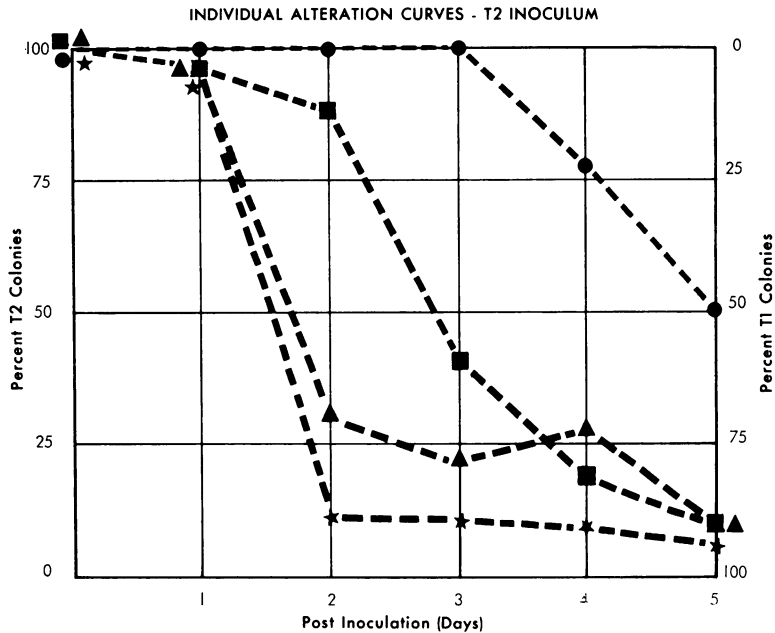


FIG. 6. Individual alteration curves—T2 inoculum. Variation in response of four volunteers to similar inocula in terms of change of colony type in daily exudate samples to alternate virulent type, considering total T1 and T2 colonies to equal 100%.

not begin to show colonial type alteration until the 4th day. Although the colonial changes occurred at different rates among the volunteers, the change seen in the FA pattern did not coincide with the colonial changes.

Animals other than man, such as mice, hamsters, rabbits, and chimpanzees, could not be infected with colonial types T1 and T2 of *N. gonorrhoeae*. Different routes of inoculation were used on mice and chimpanzees without effect, except that in some circumstances a rise in antibody titer was demonstrated.

*FA and colonial patterns after treatment.* There was a progressive decline in the numbers of extracellular FA-positive diplococci with time after treatment. No alteration was observed in the intensity of FA staining of the individual diplococci as the result of treatment. FA-positive diplococci were rarely observed at 6 to 12 hr after treatment, the length of time depending on the volunteer. No correlation was found between the rates of colonial type change and the subsequent posttreatment decline of FA-positive diplococci in exudates. The rates of decline of the number of FA-positive diplococci in exudates corresponded to the rates of declining numbers of viable cells for each volunteer. Viable diplococci were obtained from exudates of each volunteer during approximately the same period posttreatment as FA-posi-

tive diplococci could be detected extracellular to polymorphonuclear leukocytes. The number of colonies isolated from the exudates remained approximately constant for each volunteer for 3 hr after treatment and then began to decline. With some variance among volunteers, the colonial types after treatment were the same as pretreatment, until 4 to 6 hr after treatment, when they became untypable and alike in morphological appearance. No change in colonial type was observed during the treatment period up to the time they became untypable. This was expected since there was no difference reported between the colonial types of this strain in their susceptibility to tetracycline, penicillin, chloramphenicol, erythromycin, and oleandomycin (Thayer, *personal communication*).

After 720 selective transfers in vitro over a period of 35 months, the cells of strain F62 T1 colonies were still virulent for male volunteers. In each case, *N. gonorrhoeae* was reisolated from each man each postinoculation day and characterized in the same manner as after 17 months' in vitro cultivation. Two major differences were seen between the results with the 17- and 35-month inocula. First, there were more symptoms with 35-month cell inoculum than 17-month cell inoculum where symptoms were mild to nonexistent, although there were moderate amounts of puru-

lent exudate. The observation was considered significant, even though the physicians attending the subjects were not the same at 17 months (A.L.S.) and at 35 months (I.R.C.). With 35-month cells, 6 of 10 volunteers had a discharge within 24 hr, accompanied by a burning sensation. By 4 days postinoculation, these six men had all developed a tender lymph node or testes, or both, and one had a tender epididymis. The remaining four men developed discharges ranging from scant and watery to profuse and bloody by 48 hr postinoculation. No correlation was observed between the volunteers' response to inoculation and their past history of infection. Second, the daily isolate colonial type was neither colonial type T1 nor T2, but resembled a cross between the two morphologies. This morphology had been seen previously during routine passaging and had been designated colonial type T21. Progeny of these T21 colonies were predominantly T1 in colonial morphology. Serologically, most of these volunteers became reactive by the microprecipitin test within 24 hr postinoculation and remained reactive for periods ranging from 50 days to 100+ days postinoculation (Fig. 7). Such reactivity was not stable upon storage of the sera at  $-40^{\circ}\text{C}$ , and the sera became nonreactive within a few months, in contradistinction to sera obtained from clinic patients that retained reactivity for several years.

DISCUSSION

A significant result of these studies is the demonstration of a close relationship between colonial morphology of *N. gonorrhoeae* and its virulence for humans. Since the human is the only known mammal which can be infected by *N. gonorrhoeae*, the ability to identify the virulent cells and study their characteristics in vitro should expedite the development of serological tests and immunizing agents for this peculiarly human disease.

Another conclusion to be drawn from these results is that the genetic bases of colonial morphology and virulence in cells of colonial types T1 and T2 are closely related in the bacterial genome. There were many opportunities for a dissociation of these two characteristics during the 720 selective transfers since the original isolation. Many transient variations in colonial morphology within the described colonial characteristics were observed, which apparently resulted from environmental factors such as medium alterations, gas state variability, agar-medium surface moisture, and others. Occasionally, variants of T1 morphology were detected that would perpetuate themselves, indicating that a genetic change had occurred. The continued appearance of T3 and T4 colonies in the T1 and T2 lines after many selective in vitro transfers is probably the result of a genetic instability in the T1 cells rather than a selection for a coexistent cell type. Evidence for this

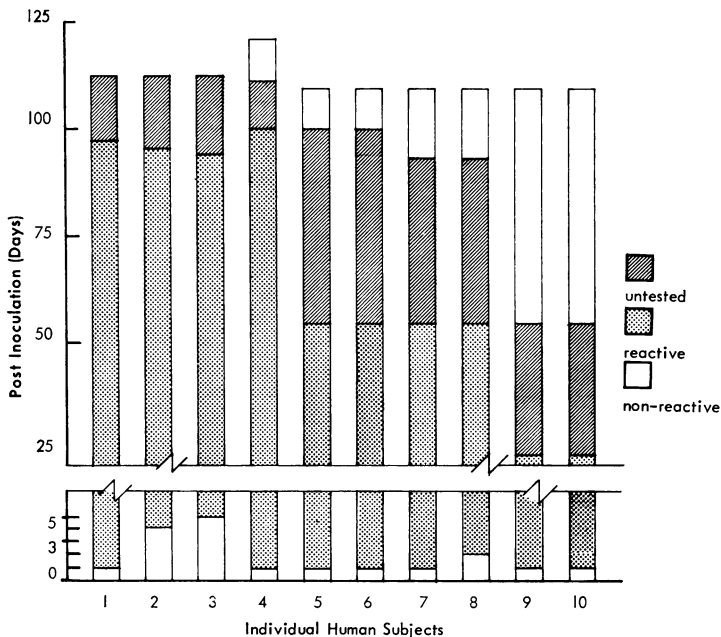


FIG. 7. Precipitin test reactivities.



hypothesis is as follows. (i) The appearance of T3 and T4 cells in T1 or T2 colonies is a discrete event, as seen morphologically by the formation of "pie segments" of T3 and T4 cells in T1 or T2 colonies; (ii) the frequency of their occurrence is a constant for each strain; (iii) their rate of occurrence was not affected by the nutritional character of the medium; and (iv) T1 colonies could not be back-selected from T4 colony lines which had been propagated *in vitro* for 2 years. In spite of these genotypic and phenotypic fluctuations, virulence and colonial morphology in T1 and T2 colonies remained inseparable.

A relationship between virulence and colonial morphology has been observed in other genera of bacteria (1, 2, 7, 14); however, in most cases, this has been either an association of colonial morphology with a degree of virulence or an association of these two characteristics in which either could vary independently of the other. In the latter category also fall several characteristics associated with virulence, such as cell surface structure, enzymes, and toxins. Of these characteristics, only the first can be associated exclusively with either virulence or colonial morphology in *N. gonorrhoeae*. The cells of virulent colonies are rough in saline, a property which may be manifested colonially by their increased convexity. Differences in cell surface structure have been regarded as reflecting variation in antigenic character (1). Differences have been observed between virulent and avirulent cells of *N. gonorrhoeae* by means of direct and indirect FA procedures, gel diffusion studies, and serum sorptions which may be associated with their antigenic structure (3, 11).

Antigenic differences between virulent and avirulent cells may be qualitative or quantitative in character; however, present evidence seems to support a quantitative distinction between virulent and avirulent cells. "Natural" antibodies have been demonstrated in persons uninfected with *N. gonorrhoeae* (3). These antibodies may result from experiences with other members of the genus *Neisseria* or members of other genera (3, 4, 17; O. Grados and W. H. Ewing, *Bacterial Proc.*, 1965, p. 53). The host does recognize the virulent cell, as seen by the development of antibodies detectable by both the indirect FA (4) and precipitin (16) procedures. Both virulent and avirulent *N. gonorrhoeae* cells possess heat-labile surface antigens which are related to their specific rather than common relationships (5) and whose presence on the cell surface depresses reactivities of the common antigens. It is possible, therefore, that the specific antigens interfere with recognition of the virulent cell. On the other hand, the virulent cells are engulfed by host phagocytic cells,

as seen by the FA examination of exudate smears. Since the toxin levels of virulent and avirulent cells are approximately equivalent and the nodes did not become tender, it may be that the rate of removal of virulent cells from the urethra to the regional lymph node is less than with avirulent cells. This could be a result of an inability of the phagocytic cells to degrade the virulent gonococci as effectively as the avirulent gonococci. This has been noted with naturally acquired gonorrheal infections (15). A recent review of phagocytosis (10) indicates that, with a few exceptions in the *Mycobacterium*, *Brucella*, and *Salmonella*, most bacteria are rapidly degraded after phagocytosis. Another author (18) feels that these exceptions could be explained by a lack of opsonization before ingestion. The elevated levels of antibodies seen in sera of the volunteers (I. R. Cohen, D. S. Kellogg, Jr., and L. C. Norins, *in preparation*) and in sera of clinic patients without parallel disappearance of virulent cells would seem to indicate that any possible opsonins might be specific, not general, in character.

The stability of the association of virulence with an easily identifiable colonial characteristic and antigenic distinctiveness in *N. gonorrhoeae* should provide a useful tool with which to study some aspects of virulence as it is related to phagocytosis in a disease peculiar to humans. The high incidence of gonorrhea, coupled with an increasing number of penicillin-resistant strains, highlights the importance of understanding the basic characteristics of the infection so as to devise more effective detection procedures, and to acquire the knowledge requisite for inducing partial or complete immunity.

#### ACKNOWLEDGMENTS

We thank Wilbur E. Deacon and M. Brittain Moore, Jr., for their support and encouragement during the 2 years required for these studies, and Charles Cravens for his conscientious and able technical assistance.

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