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Studies on Gonococcus Infection

XIII. Occurrence of Color/Opacity Colonial Variants in Clinical Cultures

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Colonial variants of *Neisseria gonorrhoeae* differing in color and opacity characteristics are distributed differently between male urethral and female cervical cultures. Colonial characteristics of cultures isolated from female cervices differ with time of isolation within the menstrual cycle and use of oral contraceptives. These differences may reflect selective forces in the ecology of the cervix, particularly proteolytic enzymes in cervical mucus and menstrual blood. Cycle changes in the characteristics of gonococci isolated from females may have implications for pathogenesis of gonococcal infections and immune reponse to the gonococcus.

The apparent coloration of colonies of *Neisseria gonorrhoeae* propagated on clear, solid media has been reported to range from light cream to dark brown-black (3, 4, 5, 10, 11, 14). Recently this variation has been explained to be based on the occurrence of surface proteins affecting the intracellular aggregation and thus the optical properties (apparent color and opacity) of the gonococcal colonies (23, 24, 25). We have studied the occurrence of these colonial variants in cultures from male urethrae and female cervices and have looked for correlation between the occurrence of particular colony forms in specimens obtained from females and the points in their menstrual cycles when the cultures were taken.

MATERIALS AND METHODS

Gonococcus. Cultures of *N. gonorrhoeae* were obtained from the urethrae of 102 males and the cervices of 104 females who visited the Salt Lake City/County Venereal Disease Clinic. The patients were not selected except for their having gonorrhea proved by culture. Each culture was nonselectively passaged from Thayer-Martin agar plate (26) onto clear typing medium with the following composition per liter: Trypticase peptone (Baltimore Biological Laboratories, Cockeysville, Md.; BBL no. 11921), 3.75 g; Thiotone peptone (BBL no. 11919), 7.5 g; K_2HPO_4 (anhydrous), 4 g; KH_2PO_4, 1 g; NaCl, 5 g; soluble starch (BBL no. 12088), 1 g; and Noble agar (Difco Laboratories, Detroit, Mich.; no. 0142-01), 10 g. After the medium was autoclaved at 20 lb/in.^{2} for 15 min and then cooled, 10 ml of IsoVitaleX (BBL no. 11876) was added to each liter before plates were poured. The plates were incubated overnight at 37°C and stored at room temperature until used. Frozen stocks of the original culture were prepared by suspending the gonococci in Trypticase soy broth (BBL no. 11767) supplemented with 20% glycerin (8), followed by storage at −70°C. All organisms were identified as *N. gonorrhoeae* by gram-stain morphology, oxidase reaction, sugar oxidation reactions, and fluorescent-antibody staining for cultures giving equivocal sugar reactions (21).

Evaluation of clinical specimens. Clinical cultures were characterized as to the color and opacity of colony types present, and an approximate percentage of the total colonies represented by each type was calculated. At the outset of the study, only color designations (extra-light, light, dark, extra-dark) were used. Subsequently, estimates of opacity (opaque, opaque-transparent, and transparent) were made using light transmitted from a polished substage mirror (25). If the earliest specimens had not received this opacity evaluation, the original first-passage frozen stock was recultivated for evaluation of colony opacity forms. Both the color and opacity characterizations were converted to a numerical value by assigning a value to each colony color and opacity form (extra-light = 1, light or dark = 2, extra-dark = 3; transparent = 1, transparent-opaque = 2, opaque = 3) and multiplying this value by the estimated percentage of each color/opacity form within the entire culture. In this way, every culture received a numerical value that indicated the central tendency for color and opacity of colony forms in that culture.

Standardized, semiquantitative estimates of trypsin sensitivity were made on the entire gonococcal population present after the first nonselective passage, as follows. A standard 20-ml amount of agar medium was poured into plates; a suspension of gonococci was made in Dulbecco phosphate-buffered saline (pH 7.2; 6) to a turbidity of 150 Klett units (Klett-Summerson colorimeter model 800-3, 12.5-mm path length, blue filter; Klett Manufacturing Co., N.Y.). This corresponds to a colony-forming unit figure of approximately 0.5×10^8 to 1.0×10^8/ml, depending on the degree of intracellular aggregation. The tip of a Dacron swab was moistened with this suspension, and excess

fluid was expressed from the swab, which was then used to streak an entire plate; a 13-mm prefilter disk (Millipore Corp., Bedford, Mass.) was placed on the streaked surface, and 0.15 ml of a 20 mg/ml solution of trypsin (type III, 2× crystallized, Sigma Chemical Co., St. Louis, Mo.) in 0.046 M tris(hydroxymethyl)aminomethane–0.015 M $CaCl_2$ was added to the disk. After 24 h of incubation in a 5% CO_2 atmosphere, the diameter of the zone of growth inhibition surrounding the disk was measured in millimeters. Initial experiments were conducted with both trypsin-TPCK (Worthington Biochemicals Co., Freehold, N.J.) and type III trypsin (Sigma) to see whether there was any detectable effect of the trace amounts of α-chymotrypsin in the type III trypsin. No difference was observed between these trypsin preparations. Additional experiments comparing type III trypsin and type III α-chymotrypsin (Sigma) indicated that sensitivity to trypsin and sensitivity to chymotrypsin are independent phenomena, in that cultures sensitive to trypsin may not be sensitive to chymotrypsin when assayed by disk sensitivity tests (data not shown). Trypsin sensitivity is defined as any zone of inhibition of growth of gonococci beyond the diameter of the prefilter disk. For purposes of the nonparametric statistical analysis presented here, the zone diameters were grouped as: no zone, resistant; ≤5-mm zone, slightly sensitive, and ≥6-mm zone, sensitive. Zone diameter is defined as total diameter − prefilter disk diameter. Clinical records for the female patients were reviewed to relate the date at which the culture was taken to the day of the patient's last menses and to ascertain the age of the patient and whether or not oral contraceptives were being taken.

Statistical analysis of data. A Wang 600 series calculator (Wang Corp., Tewksbury, Mass.) and Wang 600 series programs were used to analyze the data by several statistical techniques. Possible correlations were explored for the following groups of data: colony coloration versus trypsin sensitivity; colony opacity versus trypsin sensitivity; color or opacity of colonies from males versus color or opacity in colonies from females; trypsin sensitivity of cultures from males versus trypsin sensitivity of cultures from females; female patients' ages versus opacity of colonies in their specimens; distribution of particular colony opacity forms in the menstrual cycle; trypsin sensitivity of organisms in cultures taken at various times in the menstrual cycle.

Microscopy. A stereo microscope (StereoZoom 7; Bausch & Lomb, Rochester, N.Y.), equipped with a substage reflector having both a diffusing surface and a plane polished mirror, was used. Micrographs were obtained on Plus-X film (Kodak) with a Canon FTb camera attached to the stereo microscope.

RESULTS

Occurrence of colony color/opacity variants. Colonies having widely differing colors and opacities were first noted in gonococcal strains F62 and MS11, which have been serially passaged daily for several years. Within these strains, the entire spectrum that we recognize for color and opacity variation has been observed, as described in another report (25). These laboratory-strain preparations were utilized as standards against which the properties of strains recently obtained from clinical specimens could be compared. Although many of our initial observations were made on these laboratory strains, our observations on many strains obtained from patients with gonorrhea suggest that the optical properties observed with laboratory strains are applicable to recently isolated *N. gonorrhoeae* grown on transparent, solid medium.

Gonococcal colonies exhibit colors ranging from nearly colorless or light tan to dark brown (diffusing substage reflector) and opacities from transparent to opaque (polished-mirror substage reflector). A wide range of color/opacity forms may not be present in the initial cultures from clinical courses or in any single passage of either clinical or laboratory strains, but careful observation and appropriate selection of single colonies with subtle differences in color/opacity will yield a broad color/opacity spectrum of colonies for any strain of gonococci after a few passages. In some clinical isolates, a wide array of colony color/opacity forms were present on the first nonselective passage, even though one form might predominate. In other clinical specimens, virtually all the colonies found after one nonselective transfer to clear medium were of a single colony color/opacity type.

Colony color/opacity and trypsin sensitivity of cultures obtained from male and female patients. Initial studies suggested that isolates from males' urethrae often consisted predominantly of dark colony forms, whereas light-colored colonies were more prominently represented in cultures from females' cervices. Since the policy of the local Venereal Disease clinic is to collect only cervical, rectal, and pharyngeal cultures on females, we were unable to obtain urethral cultures from females. As a result, these and the subsequently recognized differences in gonococci present in the specimens could be attributed to the patient's sex, the anatomical source of the culture, or both. It should be noted, however, that rectal and pharyngeal cultures on both males and females are frequently dark and opaque in comparison both to the cervical isolate of the same female or to the urethral isolate of the same male, suggesting that the anatomical source of the culture may be important.

Classification of colonies in preparations of laboratory strains is relatively easy, and the categories previously described are relatively clear-cut; attempting to classify individual colonies or accurately describe color/opacity characteristics of an entire culture was much more

difficult. First, colonies of gonococci found in the initial nonselective subculture onto clear typing medium were often atypical in several ways as compared with laboratory strain preparations. The colonies in clinical specimens tended to have either a more granular appearance or a more mucoid appearance and were often very small; these colonies were often unclassifiable by Kellogg's scheme (14). In spite of this, we felt it was important to visualize as many colonies as possible that were present in the entire clinical culture and to assign a discrete, descriptive, numerical value of colonial color and opacity for each culture. After a single nonselective transfer, each specimen was also tested in a standardized disk-type assay for growth inhibition by trypsin. In this assay, a well-demarcated zone of growth inhibition was easily observed (Fig. 2). However, sometimes the results of this assay were paradoxical. An occasional clinical culture displayed trypsin sensitivity that would not have been predicted on the bases of 1) the color/opacity indices for the culture and 2) the trypsin sensitivities of homogeneous light/transparent and dark/opaque preparations derived from these specimens. Some predominantly dark/opaque colony preparations were more trypsin resistant and some predominantly light/transparent colony preparations more trypsin sensitive than were the purified opaque and transparent colony preparations derived from the original culture.

Study of gonococci isolated from 102 males and 104 females confirmed and extended the initial findings on differences in colony color noted above. Striking, easily recognized, characteristic differences were often apparent on comparing cultures from male and female sources (Fig. 1). Many female cervices yielded almost exclusively light, transparent colonies, whereas this occurred very infrequently in cultures from male urethrae. Cultures from male

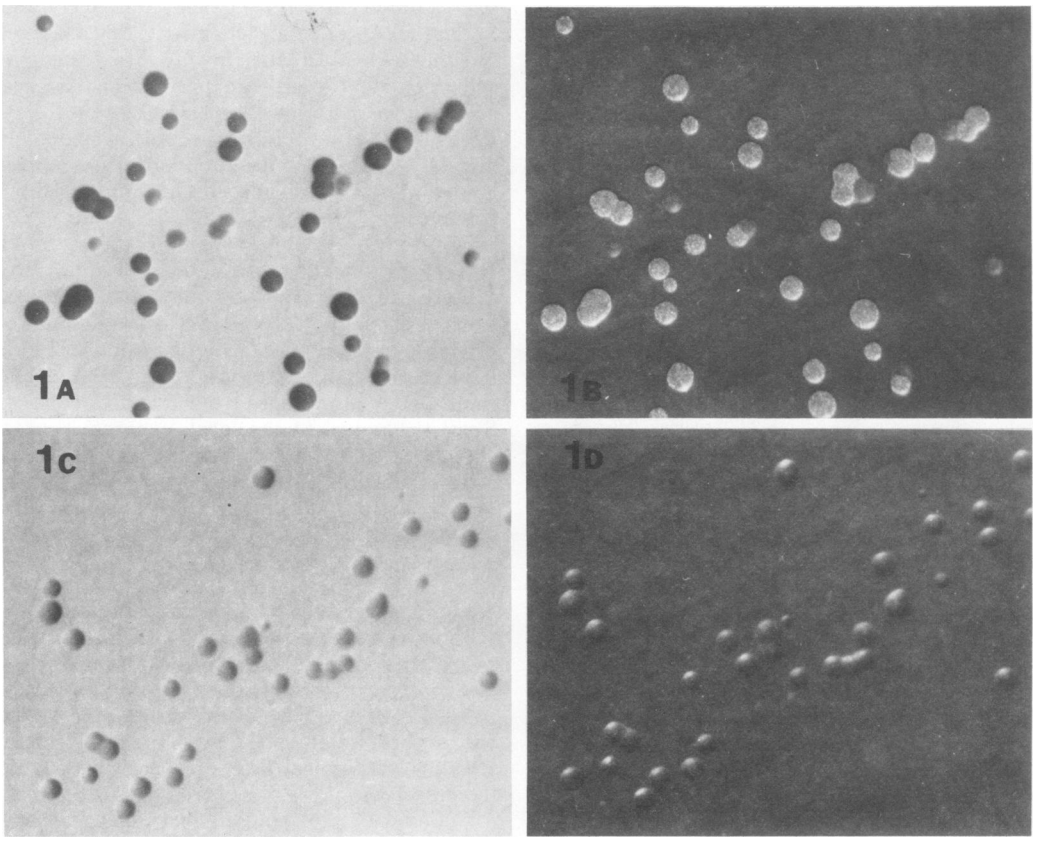

FIG. 1. *Comparison of a typical culture isolated from a male urethra (A and B) and a typical culture isolated from a female cervix at menstruation (C and D). Microscopy is by diffusing surface substage mirror (A and C) and polished plane substage mirror (B and D). The male urethral culture is dark and opaque and the female cervical culture is light and transparent.*

urethrae contained predominantly colonies that were darker and more opaque than the predominant colonies found in cultures from female cervices. On the other hand, a number of cultures from female cervices contained large numbers of dark, opaque colonies. The statistical analysis of the culture characteristics on gonococci from men and women is shown in Table 1. Cultures from male urethrae and those from female cervices differed with regard to colony color, colony opacity, and sensitivity to growth inhibition by trypsin at statistically significant levels. Somewhat different methods of analysis revealed additional correlations and intercorrelations for these culture characteristics (Table 2). Sensitivity to trypsin was significantly correlated with colony opacity but not with colony color for cultures from both males and females. Colony color was highly correlated with colonial opacity of gonococci from male patients but only approached significance for organisms from females. The reason for the seeming inconsistencies among these characteristics may be the difficulty of grading the coloration and opacity characteristics of some single colonies and in assigning a specific color or opacity value for an entire population of colonies within a given culture. This was particularly true for cultures that were intermediate in color and opacity or were of mixed dark-light, transparent-opaque composition. In addition, some cultures contained colonial forms that were both relatively light in color and relatively opaque. These light/opaque colonial forms tended to be very sensitive to trypsin.

Correlation of culture characteristics with menstrual history. Colony color and opacity as well as trypsin sensitivity of initial

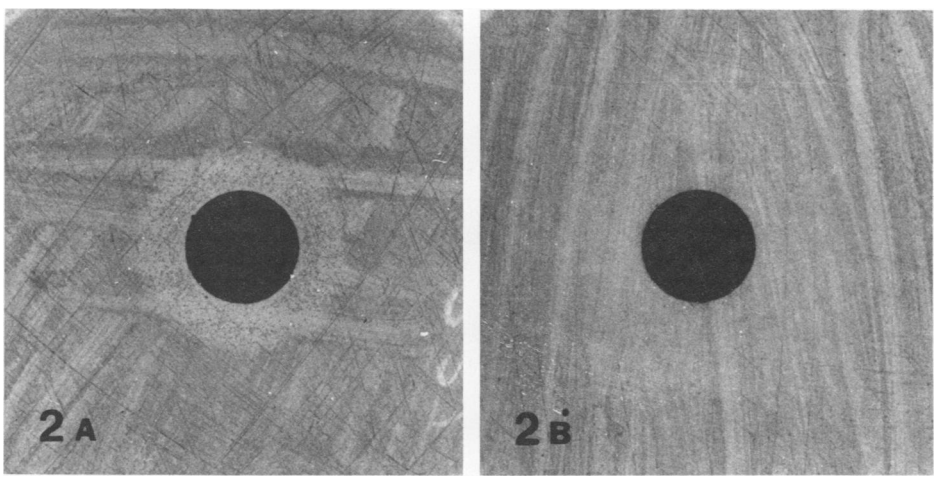

FIG. 2. *Comparison by trypsin disk sensitivity to 20 mg of trypsin per ml of (A) a male urethral culture and (B) a female cervical culture isolated at menstruation. The cultures have been stained with oxidase reagent to enhance the photography of the growth. In (A), note the trypsin-resistant colonies occurring in the zone of inhibition. Such colonies are consistently of light/transparent characteristics.*

TABLE 1. *Comparison of color, opacity, and trypsin sensitivity characteristics of gonococcal populations isolated from males and females*

Gonococcal culture characteristic	Statistical analysis[a]					
	Hosts' sex	*N*	Mean	Standard deviation	*t*	*P*
Colony color	M	102	2.00	0.345	5.002	<0.001
	F	104	1.72	0.352		
Colony opacity	M	102	1.99	0.543	2.575	$0.01 > P > 0.005$
	F	104	1.80	0.553		
Trypsin inhibition zone size[b]	M	102	5.77 mm	1.44	5.484	<0.001
	F	104	2.53 mm	0.563		

[a] Statistical analysis is presented as Student's *t* test for significance of difference between means. *P* values indicate that gonococcal populations isolated from males and females are significantly different with regard to colonial color, colonial opacity, and trypsin sensitivity.

[b] Zone size = total diameter − disk diameter.

TABLE 2. *Comparison of correlations between the gonococcal population variables*

Variables tested for correlation	Statistical analysis[a]			
	Hosts' sex	χ^2	C	P
Colony color and trypsin zone size	M	6.60	0.248	>0.3 (NS
	F	1.40	0.115	>0.8 (NS)
Colony opacity and trypsin zone size	M	49.56	0.579	<0.001
	F	24.86	0.439	<0.001
Colony color and colony opacity	M	39.96	0.532	< 0.001
	F	20.80	0.408	$0.025 > P > 0.01$ (NS)

[a] Statistical analysis used the nonparametric contingency coefficient $C = \sqrt{\frac{\chi^2}{N + \chi^2}}$ since it does not require an assumption of normality of populations or underlying continuity for the variables compared. P values indicate a lack of correlation between colonial color and trypsin zone size but a high degree of correlation between colonial opacity and trypsin zone size. Colonial color and colonial opacity are significantly correlated in gonococcal populations isolated from males, but only approach a significant correlation in gonococcal populations isolated from females. NS, Not significant.

cultures from females' cervices were also analyzed for correlation between these characteristics and the time in the patients' menstrual cycles at which the organisms were isolated. All patients were assumed to have 28-day menstrual cycles. The patients were often one-time visitors to the clinic and were frequently uncertain about the dates of their last menstrual periods, which were sometimes said to be "about a week ago." This lack of precise menstrual data would be expected to introduce considerable error into the study; however, this seems to be minimal after comparison of our data with previously published results of others (12, 16, 18). Figure 3 contains menstrual information plotted in 5-day intervals for comparison with data previously published by Johnson et al. (12). Data in the remainder of the figures (Fig. 4–6) are depicted in weekly, 7-day intervals of time during the menstrual cycle. This coarser grouping of data seems to us to be appropriate for this study which lacks exact menstrual histories.

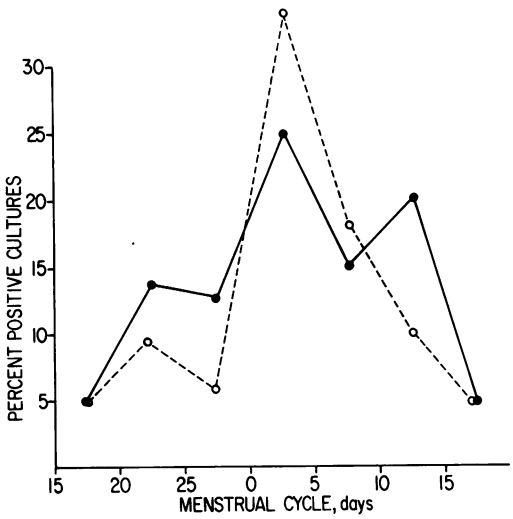

FIG. 3. *Percentage of positive cultures isolated with regard to menstrual cycle day. Graph compares the data of Johnson et al. (12) (-----) with the data obtained in this study (——). Data are plotted as midpoints of 5-day intervals. Maximum recovery at menstruation; minimum recovery at week 3.*

There was considerable variation in the number of cultures from which gonococci were isolated at various times throughout the menstrual cycles (Fig. 3 and 4), as previously noted in several studies. Our data are comparable to those presented by Johnson et al. (12) (Fig. 3) and by Lowe and Krause (18) (Fig. 4); but both of these groups of investigators found a more striking peak of positive cultures at the time of menses than we did. Regardless of the method of plotting, there was marked increase in the occurrence of positive cultures in the period surrounding menstruation (Fig. 3 and 4). This was true both for women taking oral contraceptives as well as for those who were not taking the "pill"; the former group (taking oral contraceptives) showed a slightly earlier peak of positive cultures recovered near menses than did the latter population. When plotting recovery of positive gonococcal cultures by 5-day intervals, a minor peak in recovery was seen at or just before ovulation (14 days).

The susceptibility to trypsin for gonococci isolated from female cervices varied with the menstrual cycle and the use of oral contraceptives. First, approximately half the cultures (40 out of 74) from female patients whose menstrual histories were known, regardless of contraceptive practice, were totally resistant to trypsin (no zone of inhibition) when the nonselected gonococcal culture populations were assayed

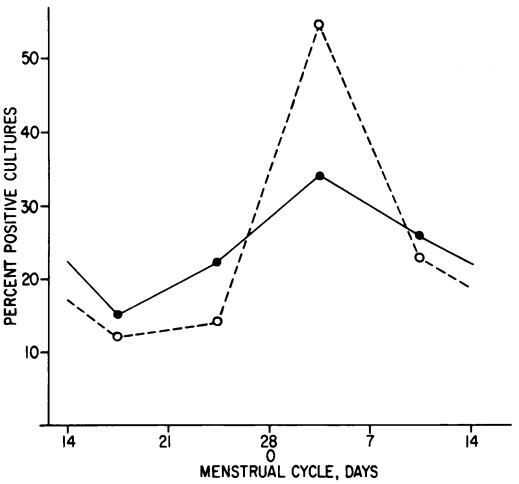

FIG. 4. *Percentage of positive cultures that were isolated plotted according to menstrual cycle day. Graph compares data of Lowe and Krause (18) (-----) with the data obtained in this study (——) and is plotted as midpoints of 7-day intervals. Maximum recovery at menstruation; minimum recovery at week 3.*

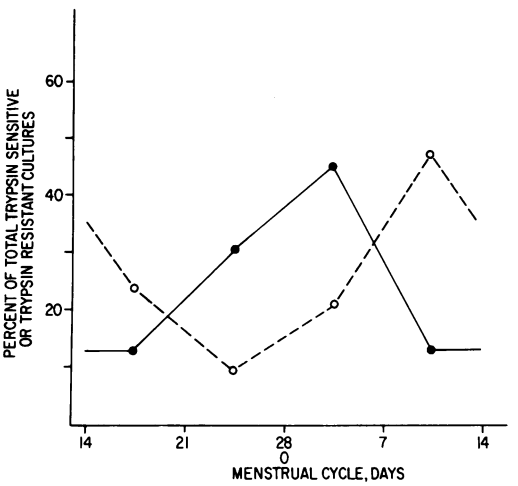

FIG. 5. *Percentage of total trypsin-sensitive cultures (-----) or trypsin-resistant cultures (——) isolated, plotted according to menstrual cycle day and graphed as midpoints of 7-day intervals. Trypsin-resistant cultures peak near menstruation and trypsin-sensitive cultures peak at midcycle.*

against 20 mg of trypsin per ml in a disk test (Fig. 2). The trypsin-resistant cultures occurred most frequently (mean, 1.0 days) during the week of menstruation and were least frequently recovered in midcycle (Fig. 5). The trypsin-resistant cultures from women not using oral contraceptives or any other form of contraception were found most frequently during week 4 of their menstrual cycles; for women using oral contraceptives, the peak of trypsin-resistant cultures was during the week of menstruation. The proportion of trypsin-resistant to total cultures was the same for both women taking the pill (18:31) and those not using this contraceptive method (17:27).

Differences in trypsin sensitivity of gonococci cultured at various points in the menstrual cycle were found when a comparison was made between patients taking oral contraceptives and those who did not take the "pill" (Fig. 6). The trypsin sensitivity of cervical cultures taken from individuals not using oral contraceptives was increased near midcycle and decreased near menstruation; the trypsin sensitivity of cervical cultures from pill-users displayed much less fluctuation during the menstrual cycle. At midcycle, cultures from women not using the pill were quite similar to those obtained from male urethrae in their mean trypsin sensitivity; male urethral cultures and midcycle female cervical cultures were not statistically different (week 2, $0.6 > P > 0.5$; week 3, $0.4 > P > 0.3$). This contrasts with the high degree of difference in cultures obtained during weeks 1 and 4 of the cycle from women not using oral contraceptives

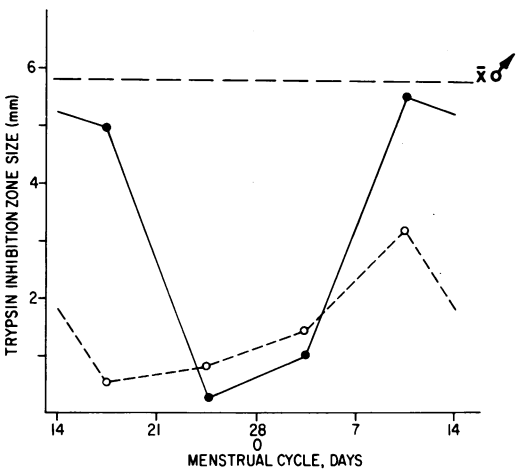

FIG. 6. *Comparison of the degree of trypsin sensitivity (zone size = total diameter − disk diameter) of cultures isolated from women taking oral contraceptives (-----) or not using any form of contraception (——). Mean trypsin sensitivity (5.77 mm) for 102 male cultures (- - - -) is included for comparison. Trypsin sensitivity of cultures obtained from women not using any form of contraception statistically approaches the mean trypsin sensitivity for cultures obtained from males only at midcycle (weeks 2 to 3). Cultures obtained from women using oral contraceptives never statistically approach the mean trypsin sensitivity for males. Data presented as midpoints of weekly intervals.*

as compared with the average of male urethral cultures (Fig. 6). At all intervals of the menstrual cycle, growth inhibition zones around trypsin disks in cultures from females using oral contraceptives were statistically smaller (week 1, $0.01 > P > 0.001$; week 2, $0.1 > P > 0.05$; week 3, $0.05 > P > 0.01$; week 4, $0.02 > P > 0.01$) than those in cultures obtained from males (mean, 5.77 mm; Fig. 6).

DISCUSSION

Several interesting findings emerge from comparison of the cultures of *N. gonorrhoeae* from males and females and from correlation of gonococcal culture characteristics with the point in the female patients' menstrual cycles at which the cultures were obtained. First, there are differences in the overall colony color and opacity characteristics of cultures from male urethrae versus those from female cervices. In this context, cultures from male urethrae tended to contain darker, more opaque colonies than did those from female cervices. Estimation of these colony or culture characteristics is, however, somewhat subjective. Assessment of trypsin sensitivity by disk assay on solid medium revealed striking differences between cultures from male urethrae and those from female cervices; this characteristic is much easier to quantitate than color or opacity of entire cultures. In general, gonococci derived from female cervices tended to be more resistant to growth inhibition by trypsin than did organisms recovered from male urethrae.

The occurrence of trypsin-resistant organisms in cervical specimens from females with gonorrhea was markedly increased at or near the time of menstruation. Positive cultures of gonococci were also obtained most frequently during this portion of the female population's menstrual cycle. Conversely, gonococci were cultivated least frequently from women in week 3 of their cycle, tended to be more susceptible to trypsin, and contained colonies that were darker and more opaque. The gonococci found in cervical specimens from women who did not use oral contraceptives appeared, for a week or so around ovulation, to have color, opacity, and trypsin sensitivity characteristics very similar to those for male-derived organisms.

Gonococci obtained from women taking oral contraceptives were quite dissimilar to male-derived organisms at all times during the females' menstrual cycles. Organisms cultured from women taking the "pill" exhibited less fluctuation of colony characteristics during the menstrual cycle than organisms cultivated from females who were not taking steroids for contraception. These findings suggest a significant host-factor component as a determiner of colonial characteristics for the gonococcal population. We do not understand which host factors are involved or the mechanisms by which the observed differences in gonococcal characteristics are produced. Our current hypotheses concerning interactions between gonococci and their human hosts responsible for the observed phenomena are as follows.

First, it is assumed that the colonial characteristics of *N. gonorrhoeae* described in this study have no influence on virulence of the organisms per se. All organisms, regardless of their colony color, etc., have been cultivated from individuals who, by definition, have gonorrheal infections. The organisms are, therefore, assumed to be virulent. Also, there is no difference in virulence of dark and light colony forms inoculated intravenously into 11-day-old chicken embryos (M. S. Blake and J. Swanson, unpublished data). Second, we cannot assume that differences observed in clinical isolates are related to male-versus-female rather than urethral-versus-cervical differences. Pharyngeal and rectal cultures are frequently of dark/opaque composition, compared to the urethral or cervical isolates from the same patient, and therefore the ecological niche of each anatomical site may be of importance in selecting gonococcal populations of varying characteristics. The demonstrated differences in trypsin sensitivity for different color/opacity forms of gonococci suggest the rather simple explanation that trypsin-like protease activity in cervical or uterine secretions bathing the cervical mucosa constitutes a selective influence for gonococci multiplying in this site. Cervical protease activity is known to increase during the luteal phase of the menstrual cycle (22), and menstrual blood exhibits high proteolytic activity (1). Increased levels of protease in the cervical milieu might select for trypsin-resistant organisms by killing relatively sensitive forms. Such a protease-related selection might be absent in males' urethrae and reduced in females' cervices that are primarily being stimulated by estrogens.

This proposed mechanism would also explain the overall greater trypsin resistance of organisms recovered during the menstrual cycle from women taking oral contraceptives, which are currently a combination of estrogenic and progestational substances. This also fits the observed similarities in trypsin sensitivity for organisms from males as compared with those isolated during weeks 2 and 3 of the cycle from females not taking oral contraceptives (Fig. 6). Combination estrogen-progestin oral contraceptives are known to have an overall effect of reducing the cyclic swings of many cervical pa-

rameters, including protease enzymes in cervical mucus (19, 22). Women not taking oral contraceptives have larger differences in cervical protease levels between midcycle and menstruation than do women taking oral contraceptives. This may account for the increased trypsin resistance of cultures isolated at or near menstruation from women not using oral contraceptives.

The proposed protease selection for trypsin-resistant gonococci is probably simplistic. Levels of many potentially inhibitory substances, including oleic acid and other fatty acids, glycogen, lysozyme, alkaline phosphatase, albumin, and globulins, vary in cervical mucus during the menstrual cycle (22). Such factors as the amount and composition of cervical mucus, cervical pH, and leukocyte infiltration of cervical and endometrial mucosae also vary in the menstrual cycle (19). How these factors might interact with cervical protease levels or actually select for gonococci with the observed characteristics is not clear. Initial experiments have suggested that, for some strains, gonococci from opaque colonies are more sensitive to oleic acid than are organisms from transparent colonies (J. James, unpublished data).

Our findings generally support the concept that variation in the recoverability of gonococci from cervices of women at different points in their menstrual cycles is due to the action of progesterone or progesterone-like substances. Progesterone has previously been shown to have a bacteriostatic effect on gonococci in vitro (7, 17, 20). This is probably responsible for the relatively reduced recovery of organisms from women who are not menstruating. Progesterone levels are decreased just before, during, and immediately after menstruation, and this might be accompanied by increased multiplication of gonococci whose growth has been partially inhibited by progesterone during the preceding portion of the menstrual cycle. This conclusion is similar to that of Koch (16), whose interesting study proves the point better than ours. It should be noted, however, that such a mechanism would be expected to yield less fluctuation in recoverability of gonococci from women taking oral contraceptives than from those relying on endogenous ovarian steroid production, and no such difference was observed.

One might speculate that the more frequent cultivation of gonococci near or during menstruation might be due to accentuated clinical symptoms of the acute or chronic gonorrheal infection during these time periods. This would explain the observed results if women with gonorrhea were more likely to attend a clinic because of their enhanced symptomatology. That explanation is not supported by the study of Johnson et al. (12), who examined patients at regular intervals regardless of symptomatology and noted the same menses-related peak in gonococcal recovery. The function of these differences may relate to the ecology of the site in which the gonococcus resides. Possession of opaque characteristics (high degree of intracellular aggregation) may have, in conjunction with pili, selective advantage in the male urethra or the male and female rectum, where it protects against the flushing action of urination or defecation. Possession of transparent characteristic (low intracellular aggregation) may confer selective advantage in the cervix where the rheological characteristics of cervical mucus would require an unaggregated organism to penetrate the gel matrix of the mucus. In addition, proteolytic enzymes of the cervical mucus may serve as a selective force by killing opaque organisms. We have recently obtained sequential cervical cultures 7 to 10 days apart on three women infected with a penicillin-resistant (β-lactamase-positive) gonococcus. These women were linked in an epidemiological chain. Sodium dodecyl sulfate polyacrylamide gel electrophoresis of the proteins of each gonococcal isolate revealed identical patterns for major outer-membrane protein; this has been proposed as a method to type gonococci (13). There were differences, however, in the proteins that have been associated with darkness or opacity (24). In all three cases, the organism isolated nearest to menstruation lacked proteins found in the organism isolated nearest to midcycle. The isolates also differed with regard to color/opacity and trypsin sensitivity as we have previously described.

The implication of cyclic changes in the gonococcus may be of profound importance. These changes in characteristics (color, opacity, proteins) may reflect changes in antigenicity and thus may give some insight into the problem of apparent lack of protective immunity to gonococcal infections. The unique nutritional requirements (15), sensitivity to antibiotics (27), and resistance to killing by serum (2) suggest that isolates from disseminated gonococcal infections are different from most other gonococci. The correlation of disseminated gonococcal infections with menstruation (9) suggests that the gonococcal isolates from these infections may also be associated with the menstruation phase of the menstrual cycle; however, we have no data to link the characteristics of these isolates and the transparent/trypsin-resistant isolates we find at menstruation.

These observations lead to the conclusion that the population of gonococci residing in a human host changes its characteristics in response to host factors. Both the male urethral and female

cervical culture comparisons, as well as the characteristics of cultures obtained at different times in the female patients' menstrual cycles, support this contention. Thus, a single strain of gonococci that infects the uterine cervix may exhibit different colony characteristics when transferred to a male host or through continuing its residence in the cervix as the hypothalamic-ovarian-uterine cycles proceed. This response of gonococcal populations to the changing ecology of the cervix may offer a new approach to the study of the pathogenesis of gonococcal infections and immune response to them.

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