CHAPTER IV

DISCUSSION

According to Kellogg et al. (55), Sparling and Yobs (57), fresh gonococcal isolates were predominated with virulent colony types: T_1 or T_2, at about 90%, and nearly all fresh isolates possessed surface pili. The present study reexamined the association of surface pili with colony typing system. Our result supported the correlation of colony type with the presence of surface pili in which only virulent colony types (T_1 or T_2) possessed pili while avirulent colony types (T_3, T_4 or T_5) did not (Table 5).Thus, colony typing system remains useful for selecting piliated gonococci from the gonococcal population. Nevertheless, our results are different from Kellogg's in that only 13 strains out of 31 freshly isolated gonococci (41.9%) possessed surface pili as observed under electron microscopy. This discrepancy might be attributed to differences in strains from each locality and/or in time lapse for culture and identification. In Kellogg's study, clinical specimens were immediately streaked on colony typing media (55).In our case, specimens were first cultured on Thayer-Martin, identified and isolated later on colony typing media, the whole process of which took 3-4 days. Piliated gonococci were thus selected by colony typing and the presence of pili were confirmed by electron microscopy before proceeding to pili

preparations.

Abundant outer membrane proteins produced by gonococci are the major problem in pili purification using ammonium sulfate precipitation technique. This problem agree with Robertson's finding that outer membrane proteins in the form of vesicles coprecipitated with pili during ammonium sulfate precipitation(114). As shown in Fig. 14 h, the crude pili preparation at first cycle was contaminated with several outer membrane proteins (molecular weight 29,000-35,000). To overcome this problem , repeat precipitation and disaggregation of the crude pili preparation up to 2 to 3 cycles could decrease the impurities, but the final yield was remarkably poor (Fig. 14j). In spite of this, pili preparation from strain S 160829 obtained from 2 cycles of repeated aggregation and disaggregation was slightly contaminated with an outer membrane protein of molecular weight 31,000 daltons which is reminiscent of a gonococcal protein II present in an opaque colony as described earlier in the Literature Review (Fig. 15f)(92). Consequently, we suggest that transparent colonies , instead of opaque ones , should be used to decrease the outer membrane contaminants during purification. On the other hand , a single pili preparation of strain S 280229 gave rise to two bands migrating closely in SDS-PAGE (Fig. 15c). It is likely that both bands are pilin since the electron micrograph of this pili preparation showed only abundant pilus filaments, with

neither outer membrane vesicles nor cell debris. This is in agreement with Salit's and Robertson's (112,114) who noted that pili preparation from a single strain appeared to give two populations of pilin with distinct buoyant densities, isoelectric points , and mobility on SDS-PAGE. These investigators suggested that this variability might reflect a change in the primary structure or a change in post translational modification of pilin subunits.

Apart from outer membrane protein contamination, another drawback is the poor yield of our pili preparations. Only five out of twelve piliated strains give enough pilin after purification,the output of which were 0.22 to 1.03 mg/ 10 g wet weight. This is 10 times lower than that of Hermodson et al.,whose pili preparations amounted to 2-10 mg/10 g wet weight of bacteria. This may be explained by two possibilities : First, the surface pili may be lost or decreased during the large scale propagation of gonococci on 200 plates of GCBS where piliated cells revert to nonpiliated state at high frequency (10^{-2}-10^{-4}/cell/generation) (121, 126, 127). Second, pili may be lost during the various steps of purification.

Electrophoretic mobility on SDS-PAGE of pilus were further analysed. By measuring the apparent molecular weights of pilin derived from 5 strains, our results confirmed that inter-strain heterogeneity exists, the molecular weight varying from 18,000 to 22,500. This result agrees with that

of Buchanan and others (38,107,110,112,114,134) who reported that pili isolated from different strains of gonococci differ in subunit molecular weights ranging from 16,500 to 23,000 when analysed by SDS-PAGE. According to Buchanan, although these pili were heterogeneous in subunit size , their morphology were indistinguishable. Likewise , our pili preparations were morphologically indistinguishable among different strains, being similar to those reported by various investigators (39,65,83,107,114,135,136) and to E.coli pili (167, 168).

Because of the poor yield of our pili preparations, only purified pili of one strain S 040629 which gave the highest yield was selected for the production of anti-pili antibody. Immunization of pili in rabbits generated antibody which reacted strongly with its immunogen as demonstrated by IHA with a titre of 1:81920 , indicating that this pilin is a good immunogen. Moreover, its antiserum afforded high specificity when tested with bacteria other than N.Gonorrhoeae by coagglutination test. Hence, the anti-pili antiserum was further used for ELISA system.

The ELISA using rabbit antiserum against gonococcal pili of one local strain developed in our study could detect homologous pili antigen, intact, and sonicated gonococci at a minimum of 0.5 ug/ml , $5x10^7$ CFU/ml, and $5x10^6$ CFU/ml, respectively. Thus, the sensitivity of the assay utilizing

intact bacteria is ten times lower than that using whole cell lysates. It is possible that in intact cells, part of the antigenic determinants recognized by anti-pili antibody may be hindered. After sonication, the hindered epitopes may be fully exposed. The sensitivity of our result was also lower than that reported by Sarafian and Young (50) who achieved at a minimum of 2.1×10^4 CFU/ml detectable whole cells with an ELISA system employing rabbit anti-whole cell. The lower sensitivity in our case might be attributed to lessened specific anti-pili IgG after anion exchange chromatography. According to Buchanan et al., concentration of IgG specific to pili which was approximately 16% of isolated rabbit IgG, would decrease proportionally when the yield of isolated IgG was low(30). Coulter et al. demonstrated that the technique could be improved by using affinity chromatography, from which the yield of specific IgG could increased five times (169).

Although the numbers of pili preparations were not large, our rabbit anti-pili antiserum seems to show little heterologous cross reaction in the ELISA system. This finding is in agreement with recent studies of several investigators, whose rabbit anti-pili had shown little cross reactivity with heterologous pili (65,114,134-136). Brinton et al. and Virji and co-workers also reported that their rabbit anti-pili antibody showed only about 10 % cross reaction (134,170). In addition, our anti-pili antibody reacted with

one particular pili possessing comparable subunit size with that of homologous pili antigen. These finding suggest that the rabbit antiserum was highly specific to homologous pili and recognized the epitope (s) located in the variable region on gonococcal pili.

Our ELISA system aimed to detect pili antigen in clinical specimen showed that the assay could only identify pili antigen in a few isolates (5 out of 102). It is conceivable that either our isolated gonococci possess heterologous pili with noncross-reacting epitopes or it lacks surface pili. To scrutinize these possibilities, all 102 gonococci were examined under the electron microscope from which 35 piliated strains were disclosed (34.3%). Nonetheless, all 102 gonococcal strains were virulent strains isolated from patients with gonorrheal infection and should have surface pili (55,57-61). Thus , the absence of surface pili in 67 strains might result from the reversion of piliated cells to nonpiliated state during storage and nonselective subcultures. This observation agrees with that of Jacobs et al. (125) and ofek et al. (68) who demonstrated that if the bacterial transfers were made nonselectively,the typical smooth type 3 and 4 colonies became predominant after three to six passages. From 35 piliated gonococci,only 4 presented positive results (11.4%) for pili antigen by ELISA. This might be accounted for by the assumption that our antipili IgG was directed against epitope (s) located in the type-

specific domain shared among the four positive strains.These findings seem to show indirectly that rabbit antiserum against pili from a single strain conferred approximately 11.4% cross reactivity among heterologous intact pili. In contrast, of the 67 nonpiliated gonococci, only one strain gave a positive reaction for ELISA suggesting the presence of pilin. The finding is in accordance with Bergstrom et al. (126), who stated that piliated cell might revert to a reverting phenotype (P^- rp^+) producing defective pilin,which could be found within the cell cytoplasm in spite of its inability to assemble into a pilus. This might account for the reactivity in our case in which whole cell lysates were examined.

According to our study,the cross reactivity of rabbit anti-pili antibody was low (11.4%) in contrast to the high cross reactivity of human anti-pili antisera (50-100%) demonstrated by Buchanan et al.(30),Brinton et al.(134), and Reiman et al. (39). The differences may be explained as follows : First, gonorrhea is a disease exclusively found in humans, so that human immune system might have evolved to be different quantitatively with respect to gonococcal antigen from the rabbit immune system. Second, humans may have exposure to the gonococcal pilus common antigen at some time in their lives, whereas rabbits have not. Third, the gonococcal pilus, may have a special affinity for human antibody-producing cells but not for rabbit. And fourth,

human being, unlike rabbits, respond to a common antigenic determinant on gonococcal pili. The detection of anti-pili in human was made use of by Lind and associates for the diagnosis of gonococcal infection in 1980(40). Nevertheless, the test could not differentiate a past infection from current gonorrheal disease.

The present study attempted to develop an assay for the detection of gonococcal antigen,especially pili, for the diagnosis of gonococcal infection. However, apart from the difficulty in pili purification, major problems emerged from the large number of different pilus immunotypes giving rise to antiserum with high specificity. Thus,with regard to the detection of antigen in clinical specimen the use of rabbit antipilus antibody to a single local strain of pili would be inadequate. The using of polyvalent anti-pili antibodies from pools of anti-pili antisera against different strains and / or immunization of purified common fragment of gonococcal pili, may be solutions to increase its capacity for pili antigen detection. On the other hand, the highly specific antipili antiserum may be most useful in developing a pilus serotyping system for N.gonorrhoeae Taxonomy and for the classification of gonococcal strains in epidemiologic analysis. Further study should improve the method of pili preparation, evaluate the ELISA system using polyvalent anti pili antisera, and examine the various immunologic types of gonococcal pili from local strains for the development of suitable assays.