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GONOCOCCAL SUBGROUPS: IDENTIFICATION, ISOLATION AND PHYSICOCHEM--ETC(U)
JAN 76 M A APICELLA, J C ALLEN DAMD17-74-C-4130

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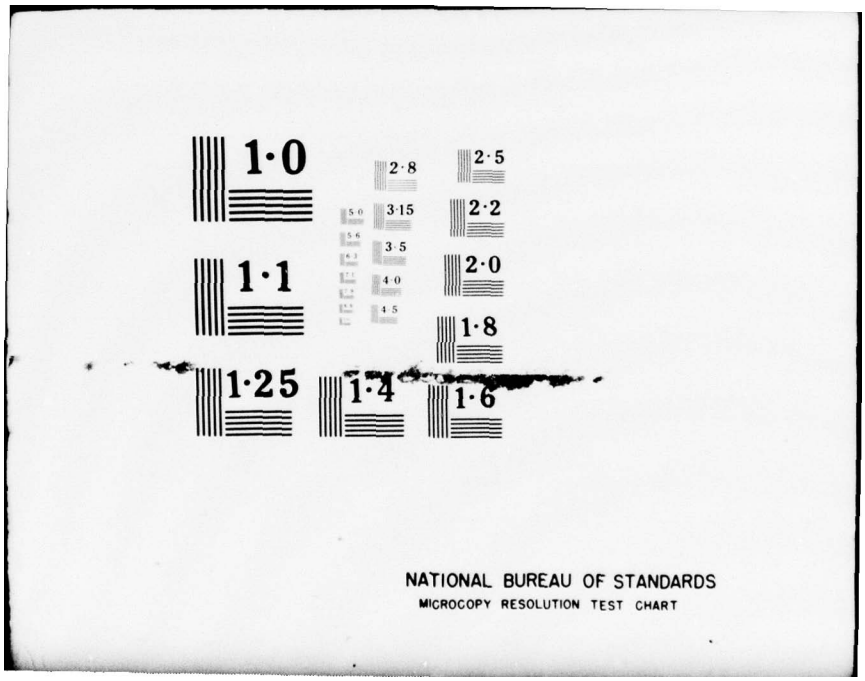
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This final progress report gives the details of studies into the development of a gonococcal serogrouping system based on a family of acidic polysaccharides isolated from gonococcal phenol water extract by DEAE chromatography. Initially, two antigenically distinct populations of gonococci were identified and designated Gc_1 and Gc_2. During the course of this study, two additional serogroup antigens designated Gc_3 and Gc_4 have been identified and isolated. All serogroup antigens share common core structures which are

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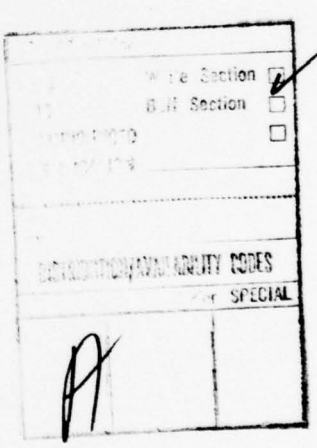
identifiable immunologically. An HAI system has been developed which can be used to detect the presence of these serogroup antigens on clincally isolated gonococci. Organisms are prepared for analysis by production of an extract which is standardized by their nucleic acid content. One hundred and sixty-three clinically isolated gonococci were studied in this HAI system and seven populations have been identified. Approximately 16.5% of the gonococci did not type for any of the four serogroup antigens or any combinations of them. The majority of strains typed for the Gc_2 group; with 40.5% of the strains in that category. The next largest group was Gc_4 and contained 16.6% of the strains. Antigen isolation studies have demonstrated the reliability of these HAI systems. During the course of cross-reactivity studies it was found that similar antigens can be isolated from group B meningococci. A method of isolation of these antigens using DEAE chromatography has been developed.



Summary

This final progress report gives the details of studies into the development of a gonococcal serogrouping system based on a family of acidic polysaccharides isolated from gonococcal phenol water extract by DEAE chromatography. Initally, two antigenically distinct populations of gonococci were identified and designated Gc_1 and Gc_2. During the course of this study, two additional serogroup antigens designated Gc_3 and Gc_4 have been identified and isolated. All serogroup antigens share common core structures which are identifiable immunologically. An HAI system has been developed which can be used to detect the presence of these serogroup antigens on clinically isolated gonococci. Organisms are prepared for analysis by production of an extract which is standardized by their nucleic acid content. One hundred and sixty-three clinically isolated gonococci were studied in this HAI system and seven populations have been identified. Approximately 16.5% of the gonococci did not type for any of the four serogroup antigens or any combinations of them. The majority of strains typed for the Gc_2 group; with 40.5% of the strains in that category. The next largest group was Gc_4 and contained 16.6% of the strains. Antigen isolation studies have demonstrated the reliability of these HAI systems. During the course of cross-reactivity studies it was found that similar antigens can be isolated from group B meningococci. A method of isolation of these antigens using DEAE chromatography has been developed.

Table of Contents

Introduction: This progress report outlines the work performed under the auspices of USAMRDC contract no. DAMD-17-C-4130 from July 1, 1974 to October 31, 1975. There were three major technical objectives in this contract. They can be summarized as follows: 1) The establishment of a gonococcal serotyping system based on two population specific polysaccharides isolated from the cell wall of the gonococcus and designated Gc_1 and Gc_2 antigens. 2) The screening of a population of clinically isolated gonococci by serotyping to determine the prevalance of these specific determinants and the identification of new population specific (Gc) antigens from strains which failed to type, and 3) studies of the immunochemical and physicochemical nature of these Gc antigens.

In this report, work performed in all three areas will be covered with the major emphasis on objectives 1 and 2. During the course of immunochemical studies with these antigens, investigations into the relationship between these antigens and other microbial species were performed. These studies indicated that similar antigens exist in Group B meningococci. Initial experiments to define this relationship will also be presented.

Final Progress Report:

A. Preliminary Studies: The studies consist of work which was in progress at the beginning of the contract period and which continued through the early phases of the contract. These investigations were directed at standardizing the method of isolation of the Gc_1 and Gc_2 polysaccharides, demonstrating the immunochemical and physicochemical characteristics of these antigens and preliminary studies into the chemical nature of these preparations. An outline of these studies is given below.

The Gc antigens of the gonococcus initially were isolated from DEAE by batch methodologies. This was replaced by use of continuous gradients over DEAE-52 columns. The following is a summary of these isolation studies subsequent to the immunochemical and physicochemical studies on the Gc_1 and Gc_2 antigens.

Gonococcal phenol-water extract for the isolation of subgroup antigen was prepared from approximately 50 grams wet weight of organisms. Prior to chromatography, 500 mgms of lyophilized phenol-water extract were dissolved in 20 ml of 0.025 N NaOH at 37C for 16 hours. The resultant suspension was titrated to pH 7 with 0.025N HCl and the sediment removed by centrifugation at 50,000 x g for 30 minutes. The supernate was removed, saved and the precipitate retreated with NaOh as described above. Prior to DEAE chromatography, the two supernates were combined, dialyzed against distilled water and lyophilized. Ion exchange chromatography to isolate subgroup antigen from alkaline digested phenol-water extract was performed with DEAE-52 cellulose equilibrated in 0.002M K_2HPO_4-K H_2PO_4 pH 6.5 in a

10 x 2 cm glass column. A gradient of increasing molarity was established in a varigrad mixer (Buchler) with 50 ml of 0.002M, 0.02M, and 0.2M K_2HPO-$K H_2PO_4$ pH6.5 in the first 3 chambers and 1.0M NaCl in the fourth and 2.0M NaCl in the fifth chamber. The column was washed with 50 ml 1N NaOH at the termination of the gradient. The sample was 25 mgm lyophilized extract in 5 ml starting buffer. Elution volumes were five milliliters and the column flow rate was 20 ml/hr.

Figure 1 shows the elution pattern obtained in the isolation of the population specific antigen from a Gc_1 organism. Immunologic detection of the antigen was accomplished by immunodiffusion with an antiserum made specific for the Gc_1 determinant by absorption with Gc_2 organisms. Antigens eluted between a conductance of 3.38×10^4 to 6.99×10^4 with the peak at a conductance of 5.03×10^4 K. Chemical studies showed that antigen elution corresponded to the major carbohydrate peak (493 nm) as measured by phenol-sulfuric acid assay. Analysis of the eluate tubes by Folin-Lowry and 280 nm absorption was negative. Studies with Gc_2 antigens resulted in an identical elution pattern and the same yield of antigen.

Figure 1

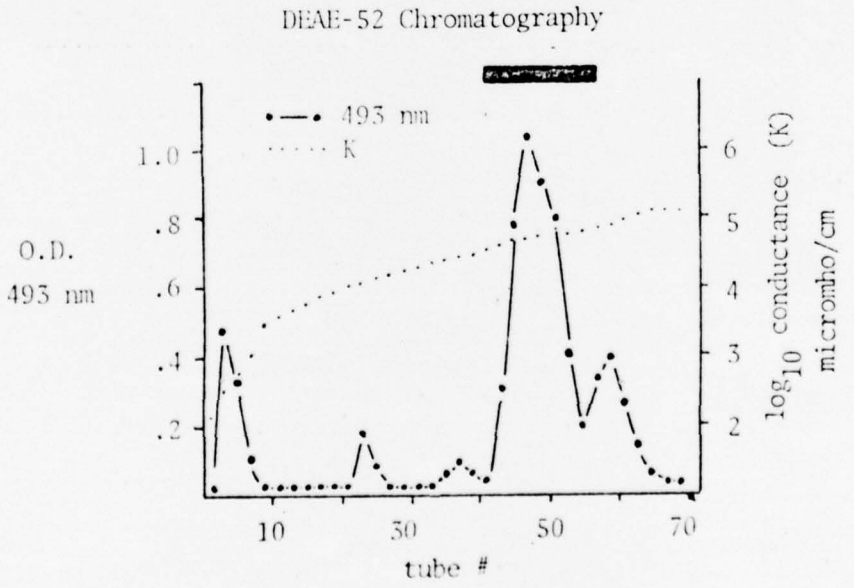


Figure 1. DEAE chromatography of Gc_1 antigen 1160. The black bar indicates the tubes containing a precipitin line with this absorbed antiserum.

Electrofocusing in acrylamide gel indicated that the isolated antigens from both populations migrated identically and contained a single component which stained only for carbohydrate with alcian blue. Unfixed gels, sectioned into 2.5 mm slices, were eluted into PBS and studied in Immunodiffusion systems using the respective absorbed antiserum. Antigen was identified in these eluates in the same region as the carbohydrate staining band. Analysis of the pH gradient of a simultaneously electrofocused gel was performed by slicing as described above and eluted into CO_2 - free glass distilled water. The antigen localized in the pH 4 - 4.5 region of the gel. Immunochemical studies indicated that the preparations after DEAE contained a single component. Figure 2 shows the results of immunodiffusion analysis with representative Gc_1 and Gc_2 antigens. The antisera were made to whole organisms and uniformly gave several lines with the alkaline digested phenol-extract prior to DEAE chromatography. After antigen isolation, only a single precipitin line could be detected. These studies demonstrated that the isolated antigens contained a common core structure in addition to the population specific determinant(s) and that both were an integral part of a single molecular species. Using the same antisera and antigens, a single precipitin line which migrated anodally, was demonstrated after immunoelectrophoresis. The immunoelectrophoretic patterns produced by Gc_1 and Gc_2 antigens were identical.

Figure 2

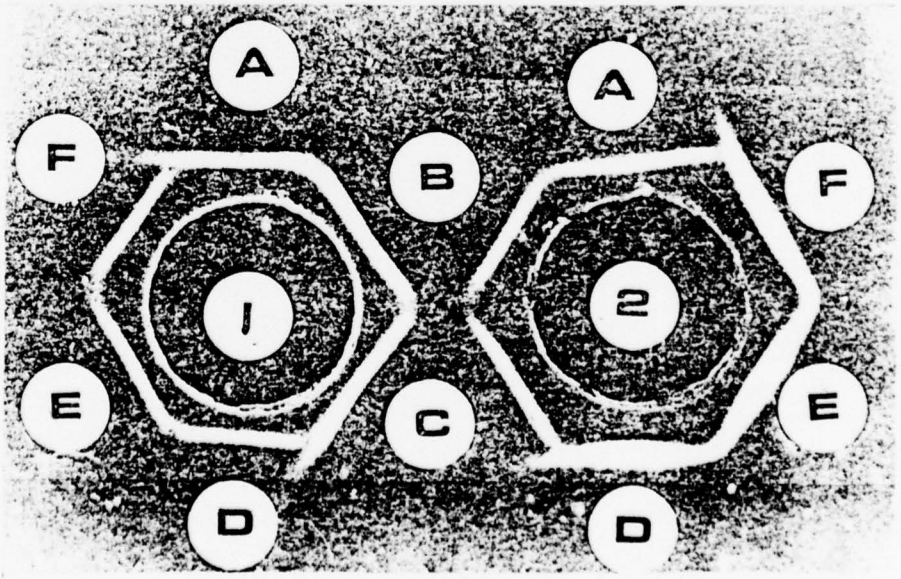


Figure 2. Immunodiffusion analysis showing the immunologically distinct populations utilizing the antigens isolated by DEAE and antisera made to whole Gc_1 and Gc_2 organisms. Wells A, B, and C contain individual Gc_1 antigens while D, E, and F contain individual Gc_2 antigens. All antigen concentrations are 1 mgm/ml PBS. Well 1 contains a Gc_1 antiserum and well 2 contains a Gc_2 antiserum.

Immunization of animals with isolated antigens in Freunds Adjuvant indicated they were less immunogenic than the determinants present on the whole organism. However, precipitating and hemagglutinating antibody could be detected in these antisera. Immunodiffusion studies demonstrated a single precipitin line with specificity for the immunizing preparation or members of its population alone. Similarly HA studies indicated that the antibody produced was population specific but at antibody titers that were sixteen-fold less than antibody produced against these determinants on whole organisms.

The antigenic integrity of isolated Gc_1 and Gc_2 antigens after various enzymatic and oxidative treatment was measured. Antigen preparations were treated with trypsin, glucose oxidase, galactose oxidase, neuraminidase, β-galactosidase, and lysozyme. None of these enzymes had any effect on the antigenicity of the core or population specific determinants in immunodiffusion or HAI studies. Treatment of antigens with sodium metaperiodate resulted in complete loss of antigenicity.

Figure 3

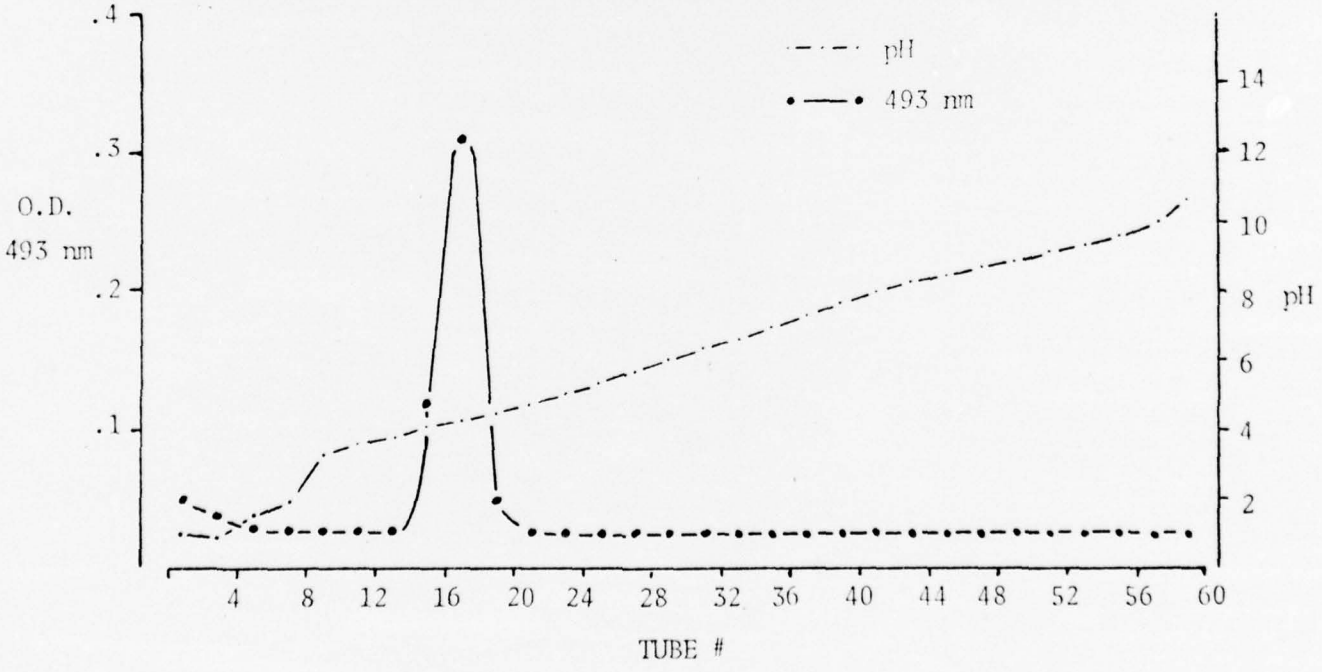


Figure 3. Elution profile of electrofocusing of Gc_1 antigen 1160. The black bar indicates the eluate tubes positive for the population specific precipitin line.

Electrofocusing studies in 0-50% glycerol gradients (figure 3) indicated that the Gc antigens were homogeneous in charge, eluting at a pI of 4.22 and 4.24 respectively. Immunologic studies after electrofocusing demonstrated the presence of both core and population specific determinants in the same peak. Chemical analysis of the column eluates were negative for protein while a single carbohydrate peak eluted which coincided with antigen elution. Analysis of the column eluates with unabsorbed whole organism antisera failed to reveal any other constituents in the samples applied to the columns.

The sedimentation coefficient of a Gc_2 antigen was determined in the analytical ultracentrifuge and the $S°_{20,w}$ was 10.08s. The antigen sedimented as a single peak. Comparative studies in 10-40% sucrose density gradients indicated that Gc_1 and Gc_2 antigens had identical sedimentation characteristics and sedimentation coefficients of approximately 10s when the formula of Martin and Ames was applied using the 10.08s Gc_2 antigen as the standard.

Molecular sieve chromatography of these antigens also indicated that they were homogeneous in size (Figure 4). Antigen eluted from P-200 as a single peak with K_{av} of 0.18. Increasing the ionic strength of the eluting buffer to 0.5M NaCl had no effect on the elution pattern. Immunologic and chemical analysis of the column elutes failed to reveal any other constituents in the isolated antigen preparations.

Figure 4

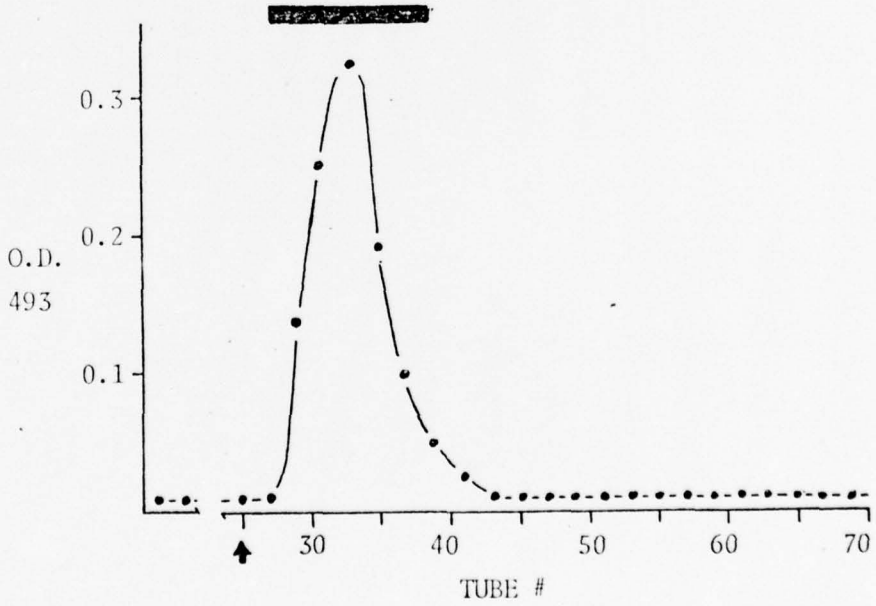


Figure 4. The elution profile of antigen 1291, a Gc_2 antigen, using P-200 chromatography. Void volume indicated by arrow. The black bar indicates the tubes in which antigen was identified by immunodiffusion analysis.

Spectral analysis of Gc_1 and Gc_2 antigens at concentrations up to 100 ugm/ml failed to demonstrate any absorption peaks over the range from 200 to 700 nm. This would indicate that contamination of these preparations by either peptides, nucleic acids or proteins if present, is minimal.

Biochemical analyses had been performed on representative Gc_1 and Gc_2 antigens. These studies indicated that these antigens were acidic polysaccharides which were essentially free of protein and were polymers consisting of hexoses and hexosamines.

B. Development of a gonococcal typing system with Gc antigens: The first priority of this study was the development of a system by which gonococci could be typed using Gc antigens. Three different systems were evaluated. These were fluorescent antibody (FA), counter-immunoelectrophoresis (CEP), and hemagglutination inhibition (HAI). All systems demonstrated applicability as potential typing techniques and useful information was gained from each. HAI was the method eventually chosen to be used to serotype a large population of gonococci for the following reasons: 1) Our laboratory had considerable previous experience with the technique, 2) it was the most efficient system as regards the consumption of reagents, particularly antisera, 3) the specificity of the system was insured by the use of erythrocytes coated with purified Gc antigens and 4) the results obtained were semi-quantitative and this has aided in the selection of strains for antigen isolation which produce large amounts of polysaccharide.

During the course of the serotyping experiments, two additional Gc antigens, Gc_3 and Gc_4, were identified. Discussion of the development of the serotyping system will include these two antigens as well as the original Gc_1 and Gc_2 antigens. In a later section of this report, the immunochemical and physicochemical properties of the Gc_3 and Gc_4 antigens will be discussed.

HAI Serogrouping: In order to incorporate gonococcal strains into the HAI systems for the serogroup analysis, an alkaline extract was produced from each strain. Three chocolate plates were heavily streaked with gonococci. After overnight growth at 35C under 5% CO_2, the organisms were harvested and suspended in 2 ml of 0.1 NaOH at 37°C. When the solution became opalescant (about 4 hours), the extract was titrated to pH 7 with 0.1 N HCl. Pronase was then added to a final concentration of 10 µgm/ml and the extract digested for 1 hour at 37C. The pronase was destroyed by placing the extract in a boiling water bath for five minutes. After cooling under tap water, the extracts were concentrated by precipitation with two volumes of cold acetone. The precipitate was collected by centrifugation at 1500 x g, dried in a stream of air, and reconsitiuted in 0.5 ml of PBS. Any undissolved precipitate was removed by centrifugation in a Serofuge, for five minutes. The extracts were standardized and frozen at -70C until studied.

To insure that the final extract preparations contained uniform concentrations of gonococci, the wet weight of the organisms used in each was determined and the final volume adjusted to 400 mgm gonococcal per ml. Initially, this was done by weighing the organisms. Subsequent studies demonstrated that an optical density of 25 units at a wavelenght of 260 nm corresponded to this concentration of gonococci. Figure 5 shows an absorption spectra in the ultraviolet range of atypical gonococcal extract. All extracts were standardized in this manner prior to testing. To accomplish this standardization, 50 lambda of extract was diluted to 2.5 ml in PBS. The optical density at 260 nm was determined in 1 cm quartz cuvettes in a Hatachi 190 spectorphotometer. The final extract volume was determined by the following formula:

$$\text{Final Volume} = \frac{\text{O.D. 260 nm}}{0.50} \times 0.45 \text{ ml}$$

The O.D. 260 nm represents the optical density of the 1:50 extract dilutions and the 0.45 ml represents the volume of the extracts after fifty lamda is removed for standardization. The constant 0.50 represents the optical density of a 1:50 dilution of 400 mg/ml of organisms.

Figure 5

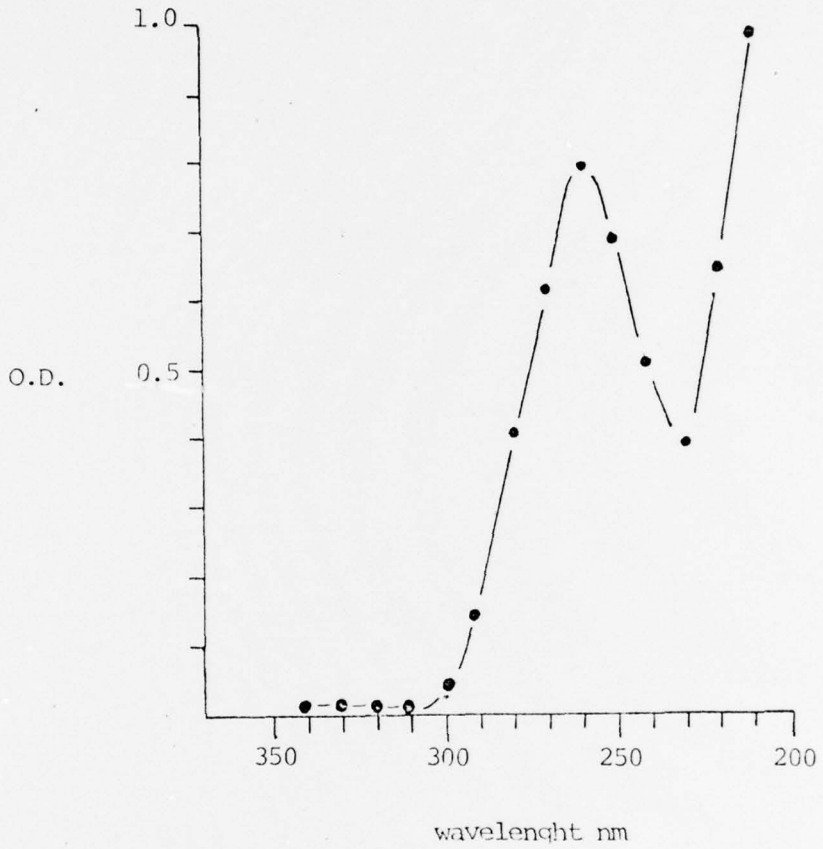


Figure 5. Spectral analysis of gonococcal extract from Gc_1 strain 1342. Absorption maxima occur in the 260 nm range corresponding to nucleic acid absorption and below 220 nm. The 260 nm wavelength was chosen for extract calibration.

Table 1

Sensitivity and specificity of the four serogroup HAI systems using serogroup antigens.

Serogroup HAI System	Inhibitor Concentration µgm/ml			
	Serogroup Antigen			
	1342 (Gc_1)	1291 (Gc_2)	4505 (Gc_3)	1108 (Gc_4)
Gc_1	16	>1000	>1000	>1000
Gc_2	>1000	4	>1000	>1000
Gc_3	>1000	>1000	8	>1000
Gc_4	>1000	>1000	>1000	1

These extracts were incorporated into four HAI systems which were specific for the respective serogroup systems. These HAI systems employed sheep erythrocytes coated with Gc antigens. The antisera used as agglutinators were made to whole gonococci and absorbed to make them specific for the respective serogroups. Table 2 demonstrates the specificity and sensitivity of these HAI systems using purified antigens.

Figure 6

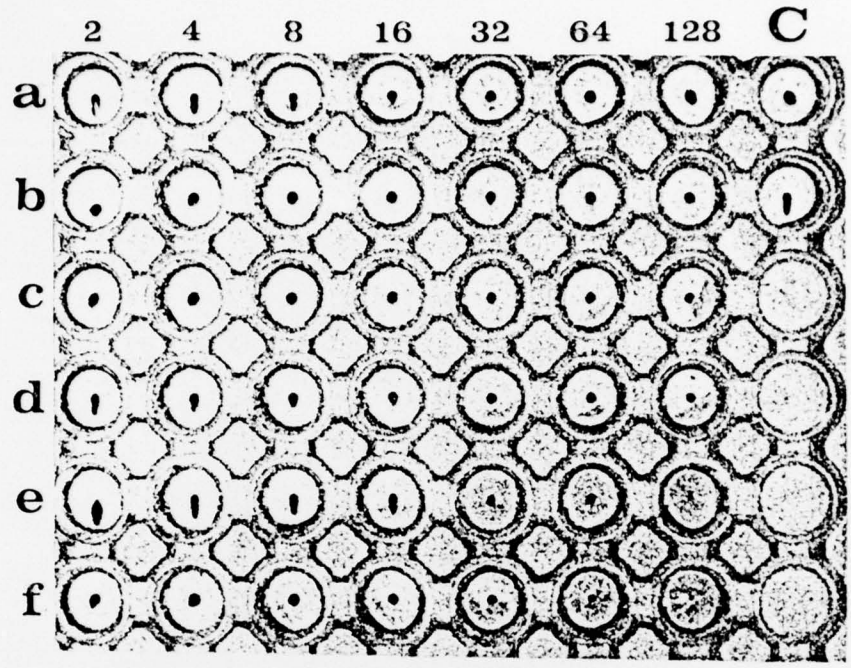


Figure 6. Microtiter HAI plate using strain extracts in Gc_1 system. Serial dilutions are dipicted across horizontal axis. Well C-A contains an agglutinating control and Well C-B, a non-agglutinating control. Rows A, B, and C contain extracts from prototype strains 1342 (Gc_1) 1291 (Gc_2), and 4505 (Gc_3). Well D, E, and F contain extracts from strains 256, 352 and 1123. Inhibition is seen in row A to a titer of 1:8, row D 1:4 and row E to 1:16.

The incorporation of extracts into these HAI systems did not effect the specificity of the systems. Figure 6 shows a photograph of a typical HAI microtiter plate demonstrating a study using extracts from standard strains for three serogroups and unknown strains. As can be seen, the only standard strain which inhibited (inhibition is manifest by the falling button) was the Gc_1 strain 1352 at a titer of 1:8.

C. Serogroup testing of clinically isolated gonococcal strains.

Extracts were prepared by the method just described to 163 consecutively isolated gonococcal strains and incorporated into the serogroup HAI systems. Table 2 gives the serogroup distribution of these strains.

Table 2

Serogroup distribution of 163 gonococci by HAI analysis

Group	No.	%
Gc_1	11	6.7
Gc_2	66	40.5
Gc_3	12	7.4
Gc_4	27	16.6
Gc_{1-3}	13	8.0
Gc_{2-4}	5	3.1
Gc_{1-4}	2	1.2
NG*	27	16.5
Total	163	100

NG = Nongroupable

Seven serologic catagories of strains were identified. Seventy-one percent (116/163) of all strains typed for only one serogroup of which 57 percent (66/116) typed for the Gc_2 serogroup. Figure 7 shows the titer distribution of the 116 strains in these four serogroups. The median titer for Gc_1 was 1:8, for Gc_2 was 1:8, for Gc_3 was 1:16 and Gc_4 was 1:16. Nine strains had titers greater than 1:128. The highest titer was 1:2048 and occurred in 3 strains in the Gc_4 system. Twenty strains typed for more than one serogroup. In order to confirm that this mixed pattern was not due to strains containing organisms of two different serogroup populations, they were replated and 10 individual colonies isolated from each parent strain. These colonies were used to produce extracts which were studied in the serogrouping systems. For each of the twenty strains, all colonial isolates demonstrated the same serogrouping pattern as the parent strain and typed for two serogroups.

Figure 7

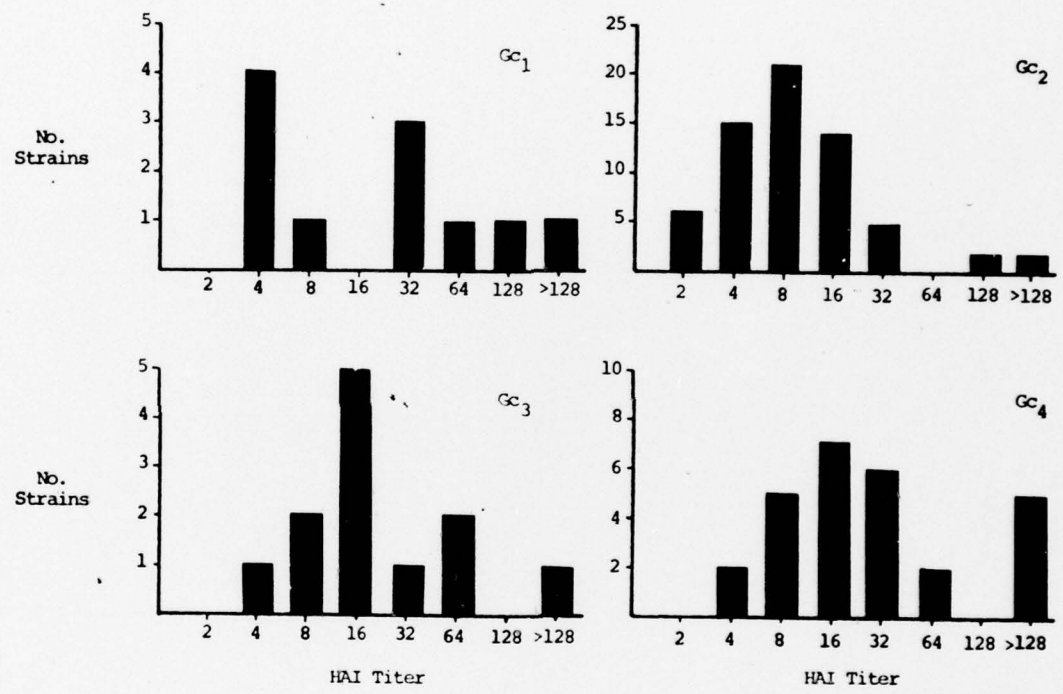


Figure 7. Frequency distribution of 116 gonococcal strains.

In order to confirm the reliability of the serogrouping systems, antigens and antisera were made to strains from each serogroup and compared in a series of immunologic studies with the standard reagents (antisera and antigen) of their own serogroup. A total of sixteen strains were studied, four in each serogroup. To test the reliability of low titers, only strains with HAI titers of 1:16 or less were selected. Figure 7 shows the results of a typical immunodiffusion study of the four antigens isolated from the Gc_2 serogroup. These are compared to the Gc_2 antigen 1291. The antiserum was made to strain 755. As can be seen a single line of indentity is present between the four antigens and the standard. When the Gc_2 antiserum 1291C is used, an identical pattern is obtained.

Figure 8

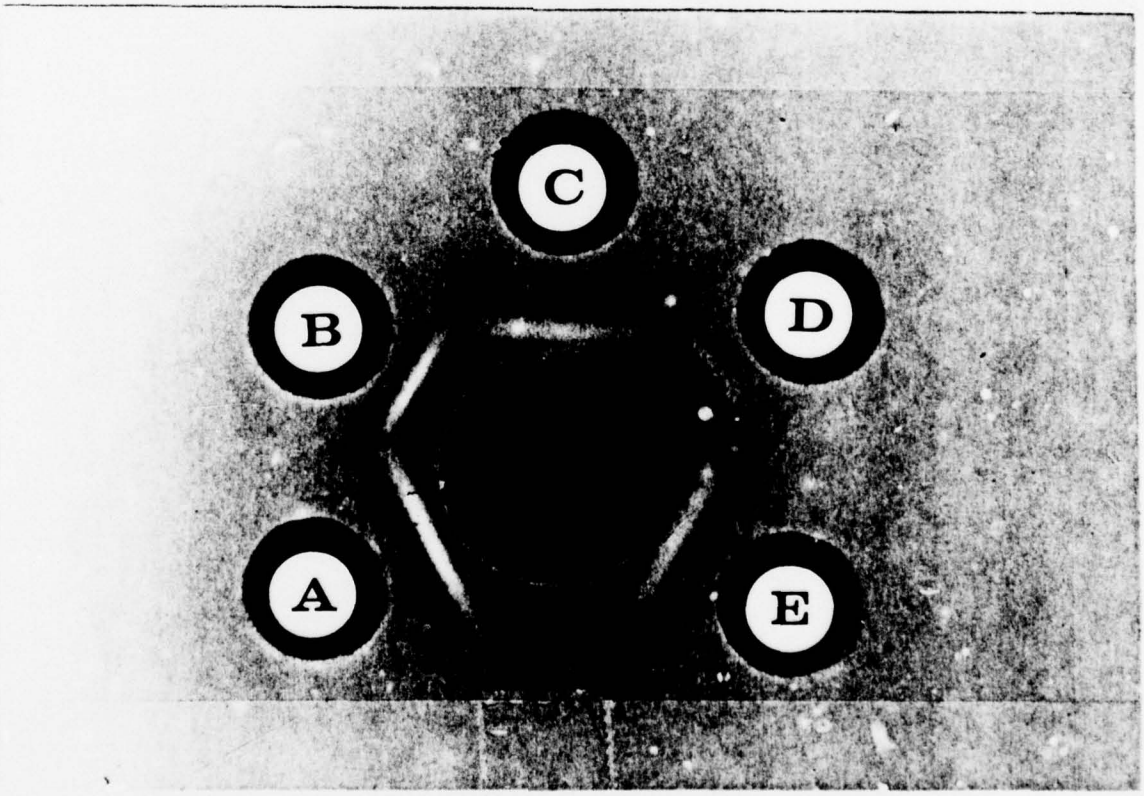


Figure 8. Immunodiffusion analysis of serogroup antigen isolated from Gc_2 strains 755, 877, 1194, and 6398 in wells B, C, D and E. Well A contains Gc_2 antigen 1291 the serogroup standard. All antigen concentrations are 500 µgm/ml. The center well contains antiserum produced to strain 755.

All sixteen antigens were used as inhibitors in the standard HAI systems. Table 3 shows the results of this study. Inhibition occurred only in the homologous systems. The range of inhibition was from 64 µgm/ml to 4 µgm/ml. Studies were also instituted to develop serogroup HAI systems with these antigens and their antisera. One pair was chosen from each serogroup. Ten extracts which had been typed with the standard HAI systems were selected and used as inhibitors in these systems. The results were compared with those obtained in the standard serogroup system. The new HAI systems were inhibited only by the same extracts which inhibited their own serogroup HAI.

At present, 27 strains could not be classified with these serologic methods. Twelve of these strains have been studied by production of antisera and attempts at serogroup antigen isolation. These antisera did not produce antibodies in precipitin or hemagglutination systems to the four known serogroups. Using these antisera, new serogroup antigens have not been identified on these strains.

D. Gc antigens identified during this study, their physicochemical and immunochemical properties.

As mentioned previously, two additional population specific antigens have been identified during the course of this study. These have been designated Gc_3 and Gc_4. The Gc_3 antigen was isolated from a strain which failed to produce Gc_1 or Gc_2 antigens based on immunodiffusion analysis. The Gc_4 antigen was isolated from a strain which failed to type in the Gc_1, Gc_2, and Gc_3 HAI systems.

Figure 9 and 10 demonstrate a series of immunodiffusion studies which show the specificity of the different serogroup systems including the Gc_3 and Gc_4 antigens. The antisera used in these experiments were made to whole organisms representative of the respective serogroups. This antisera was then absorbed with purified Gc antigen from another serogroup to remove the core specificity. These same absorbed antisera and antigens were the reagents used to construct the serogroup specific HAI systems for the detection of the respective Gc antigens in the gonococcal strain extracts.

Physicochemical studies with the Gc_3 and Gc_4 antigens indicated that they have properties identical to the Gc_1 and Gc_2 antigens. Isoelectric focusing in a 0 - 50% glycerol gradient using pH 3 - 6 ampholine buffers indicated that the pI of the Gc_3 and Gc_4 antigens are 4.20 and 4.26 respectively. Both core and serogroup specificity were present on the antigens isolated from the Gc antigen peaks after electrofocusing and no other sample components were identified in the eluate from these columns.

Figure 9

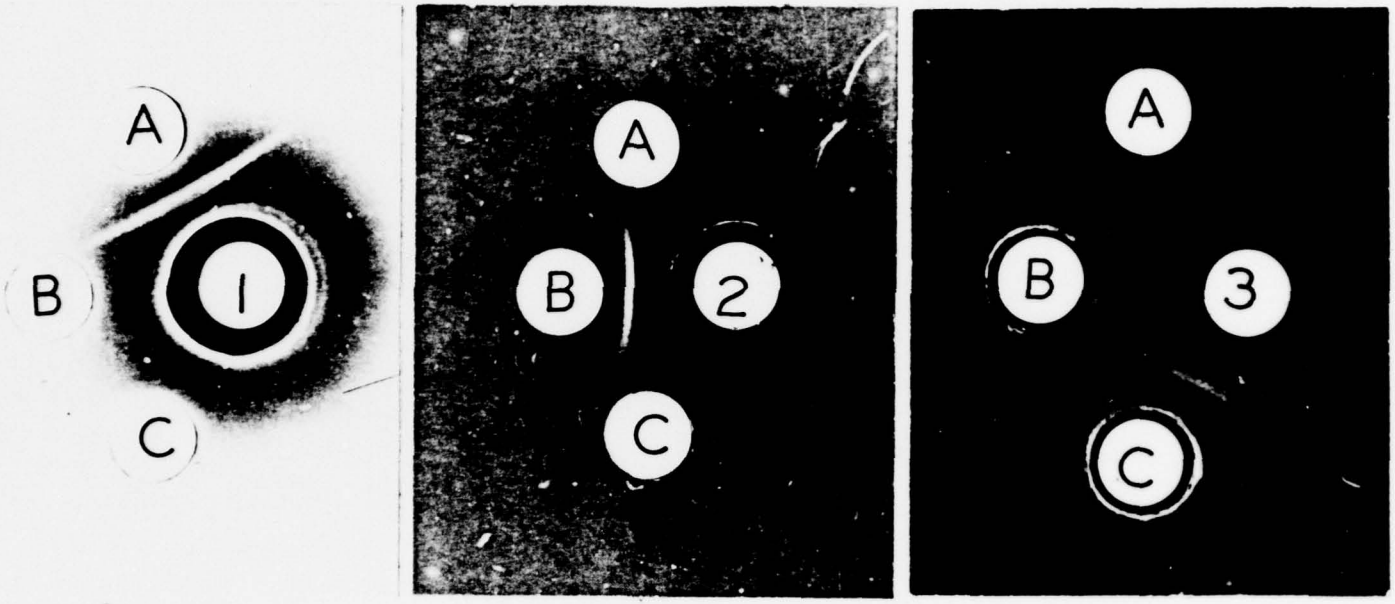


Figure 9. A composite of 3 immunodiffusion studies demonstrating the specificity of the serogroup systems. Well A contains Gc_1 antigen 1342, Well B contains Gc_2 antigen 1291, and Well C contains Gc_3 antigen 4505. Each antigen concentration is 500 µgm/ml. Wells 1, 2, and 3 contain absorbed Gc_1, Gc_2, and Gc_3 antisera.

Figure 10

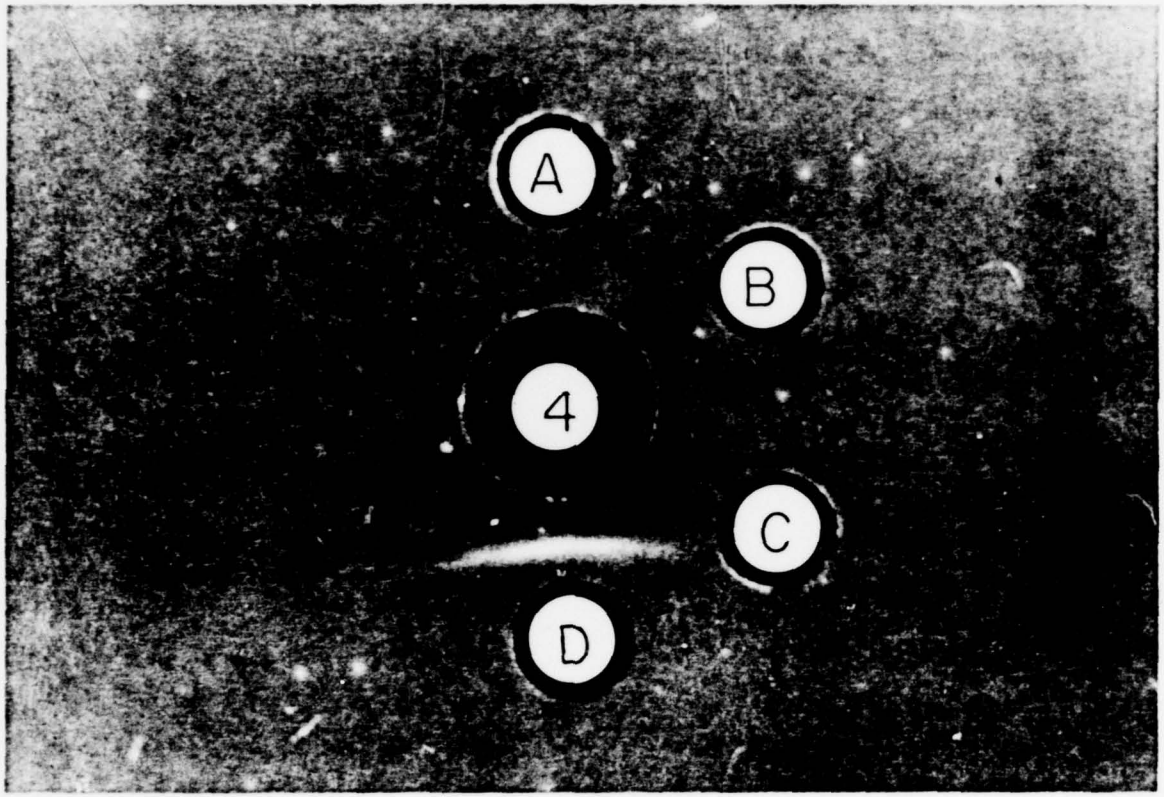


Figure 10. Immunodiffusion study demonstrating the specificity of the Gc_4 system. Wells A, B, and C are identical to figure 9. Well D contains Gc_4 antigen 1108 at 500 µgm/ml. Well 4 contains absorbed Gc_4 antiserum.

The molecular size of the Gc_3 and Gc_4 antigens is similar to that previously described for the Gc_1 and Gc_2 polysaccharides. They elute from P-200 with a K_{av} of 0.15. Enzymatic and oxidative studies of these new serogroup antigens indicated that their antigenicity is resistant to destruction by proteolytic enzymes, but completely removed by treatment with sodium metaperiodate. Studies are now underway to determine the effect of glycolytic enzymes such as mixed glycosidase on these antigens.

Chemical studies are in progress on the Gc antigens. Amino acid analysis of all of the four serogroup prototype antigens has been completed using a Beckman 120C amino acid analyzer after 24 and 48 hours of hydrolysis at 110 in 6N HCl. Amino acids accounted for between 0.78% (Gc_4) to 0.27% (Gc_1) of the applied antigen samples. Nitrogen analysis indicated that the Gc_1 and Gc_2 antigens contained approximately 2.0% nitrogen. Colorimetric analysis of the Gc_1, Gc_2, Gc_3, and Gc_4, antigens for sialic acids, hexuronic acids, pentoses, heptoses, and deoxypentoses were negative. The following chemical studies were performed only on the Gc_1 and Gc_2 antigens.

Using the anthrone method, approximately 25% of the Gc_1 and 20% of the Gc_2 antigens could be accounted for as hexoses, respectively. To determine the hexosamine content, multiple hydrolysis conditions were studied. Optimal hydrolysis occurred at 8 hours in 2N HCl at 100 C in vacuo. Under these conditions, 15% of the dry weight of the Gc_1 and 20% of the Gc_2 antigens could be accounted for as hexosamines by Elson-Morgan analysis. Table 4 shows the results of ion exchange and gas chromatographic analysis of these antigens for their carbohydrate components.

Table 4

Carbohydrate Analysis
Gonococcal Serogroup Antigens

	mgm/100 mgm antigen	
	Gc_1	Gc_2
	strain 1342	strain 1291
glucose	11.4	8.9
galactose	12.3	9.5
glucosamine	6.6	11.4
galactosamine	3.3	none present
glucosamine-6-PO_4	4.5	5.2

Ion exchange chromatography with the amino acid analyzer for amino sugars has been done on hydrolysates of both types of subgroup antigen, and a major difference has been identified. Both Gc_1 and Gc_2 antigens have been found to contain galactosamine. Another peak is also present in the chromatogram of antigens of this subgroup and it has been tentatively identified as galactosamine-6-phosphate. We are now attempting to confirm this by the preparation of authentic standards. Gas liquid chromatography for hexoses indicates that both subgroup antigens contain glucose and galactose in equimolar concentrations. The quantitative release of hexoses after hydrolysis has always been greater from the Gc_2 antigens while the release of hexosamines has always been greater from the Gc_1 antigens. Repetition of these hydrolysis experiments under precise quantitative conditions are now underway to define these differences exactly. We are now able to account chemically for approximately 40% of the Gc_1 antigen and 35% of the Gc_2 antigen. A wide range of hydrolysis conditions have been studied and additional studies are planned. Finally, the relatively low yield of hexosamines in antigens which based on their nitrogen content should consist primarily of amino sugars has prompted us to search for unusual amino acid sugar derivatives such as amino-hexuronic acid derivatives and to look for the more acid labile hexosamines such as 4-amino-4 deoxy glucose. More complete chemical analysis of the Gc_3 and Gc_4 antigens are now in progress.

E. *Cross-reactivity Studies: Identification of a non-capsular cell-wall polysaccharide from group B meningococci similar to the Gc antigen of the gonococcus:* During the course of investigations into cross-reactivity of the Gc antigens with other microbial species the following experiments were performed. Antisera, to a wide range of microbial species or microbial antigens were studied in immunodiffusion systems against the Gc antigens or in passive hemagglutination systems (HA) to cell coated with the four Gc serogroup antigens. Table 5 demonstrates the results obtained in these studies. As can be seen, among 31 antisera tested, cross-reacting antibody was demonstrated only in antiserum made to several strains of group B meningococci. The following preliminary studies have been performed in an attempt to elucidate this relationship between group B meningococci and the Gc antigens of the gonococcus.

Ten group B strains which had been isolated from patients with serious CNS infections or sepsis were selected, grown on chocolate plates and alkaline extracts made to them by the method described for gonococci. These meningococcal extracts were then incorporated into the four Gc serogrouping systems as inhibitors. Four of the group B extracts inhibited the Gc_2 serogrouping system at dilutions of 1:4, 1:8, 1:8, and 1:32, respectively. No inhibition was seen with any of the ten extracts in the three other gonococcal serogrouping systems. To determine the nature of the cross reacting determinant(s), isolation of the responsible antigen(s) was under taken by the method used for the GC antigens. DEAE chromatography was done on the alkaline

Table 5

Results of immunodiffusion and passive hemagglutination studies with antisera to other bacterial species.

Antiserum	Source	Precipitin line	HA
E. coli OB poly A	Difco	-	-
E. coli OB poly B	"	-	-
Shigella poly C-2	"	-	-
" " A	"	-	-
" " A-1	"	-	-
" " B	"	-	-
" " C	"	-	-
" " C-1	"	-	-
" " D	"	-	-
Klebsiella type 1	"	-	-
" " 2	"	-	-
" " 3	"	-	-
" " 4	"	-	-
" " 5	"	-	-
" " 6	"	-	-
E. coli OK poly C	"	-	-
Salmonella O poly A	"	-	-
Salmonella Vi	"	-	-
Hemophilus poly	"	-	-
Brucella mellitinois	"	-	-
" suis	"	-	-
Brucella poly AMS	"	-	-
Listeria O	"	-	-
Yersinia tularensis	"	-	-
Herellea Vaginocula	"	-	-
Pneumococcal poly	Statens	-	-
N. mening. A	Own	-	-
N. mening. B	"	Gc_2	Gc_2
N. mening. C	"	-	-
N. Flava	"	-	-
N. sicca	"	-	-

treated phenol-water extract of group B meningococcal strain B-47, and Gc_2 antisera 1291C was used to detect the antigen in eluate tubes. The antigen eluted at an ionic strength of between 3.4 x 10^4K to 5 x 10^4K which is identical to the Gc antigens. Immunodiffusion studies comparing B-47 with the standard antigen for the Gc serogroup in which it typed indicated they were identical. Such an immunodiffusion study can be seen in figure 11. Like the Gc antigens, this meningococcal antigen is a macromolecule of substantial size. Figure 12 shows the elution of this antigen over a Sepharose 4B column. The antigen again was detected with 1291 C antisera and eluted just after the void volume of this column. The Gc antigens eluted from this column in a similar region. Electrofocusing studies have been completed in a 0-50% glycerol gradient and reveal that meningococcal antigen from strain B-47 has a pI of 4.19. Previous studies with gonococcal Gc antigens indicate that they have isoelective points in the range of 4.20 to 4.26. Two of the remaining three group B meningococcal strains which inhibited the gonococcal Gc_2 serogroup have been studied by antigen isolation and in the same studies as described above. All yield antigen identical to the Gc_2 standard 1291 and all had similar elution characteristics in DEAE, Sepharose 4B, and isoelectric focusing.

Chemical and immunologic studies have indicated that these antigens are unrelated to the group B capsular polysaccharide. They are not detected by CDC group B antiserum in immunodiffusion or hemagglutination systems and their ability to inhibit the Gc_2 HAI system is not altered by neuraminidase treatment. (The group B polysaccharide has been described as being neuraminidase susceptibile.) Chemical analysis of these antigens indicated that they are polysaccharides containing less than 1% protein based on amino acid analysis. Specific chemical definition of these antigens is not yet available, but the sutdies are in progress.

In addition to studies described above of the four meningococcal polysaccharides which inhibited the Gc_2 systems, antigen isolation and antisera production has been accomplished on four group B meningococcal strains which failed to inhibit any of the gonococcal Gc serogrouping HAI systems. In this group of four strains, two different polysaccharide are immunologically distinct from each other and the antigens isolated from the meningococci which inhibited the Gc_2 system. These antigens have been produced by the methods described above for Gc antigens and were detected by antisera made to the parent group B strain. Figure 13 shows an immunodiffusion plate recently completed with these antigens and absorbed antisera. Three specific immunodiffusion systems have been established. A single line is present only between homologous antigens and antisera.

Figure 11

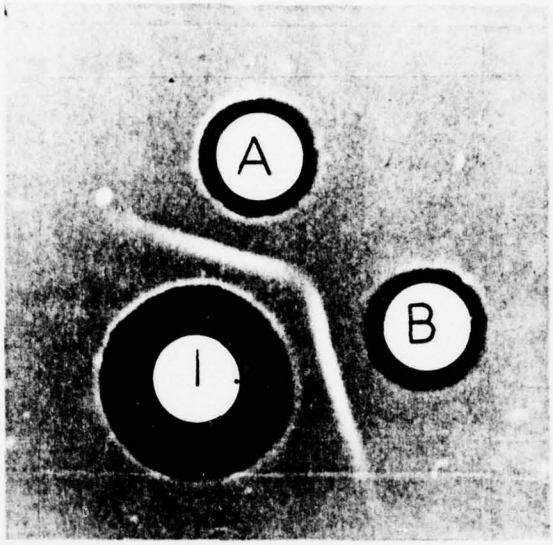


Figure 11. Immunodiffusion study comparing Gc_2 antigen 1291 (well A) with antigen from strain B-47 (well B). Well 1 contains antiserum 1291C.

This would indicate that there are at least 3 group B meningococcal serotypes based on this non-capsular cell wall polysaccharide system. Studies are now in progress to establish HAI systems to these serotype antigens in a fashion similar to the gonococcal HAI systems.

Thus, our studies with gonococcal serogrouping have indicated that similar and in some instances identical, antigens exist in the cell wall of group B meningococci. At least 3 different antigenic populations of these polysaccharides have been identified in group B meningococci.

Figure 12

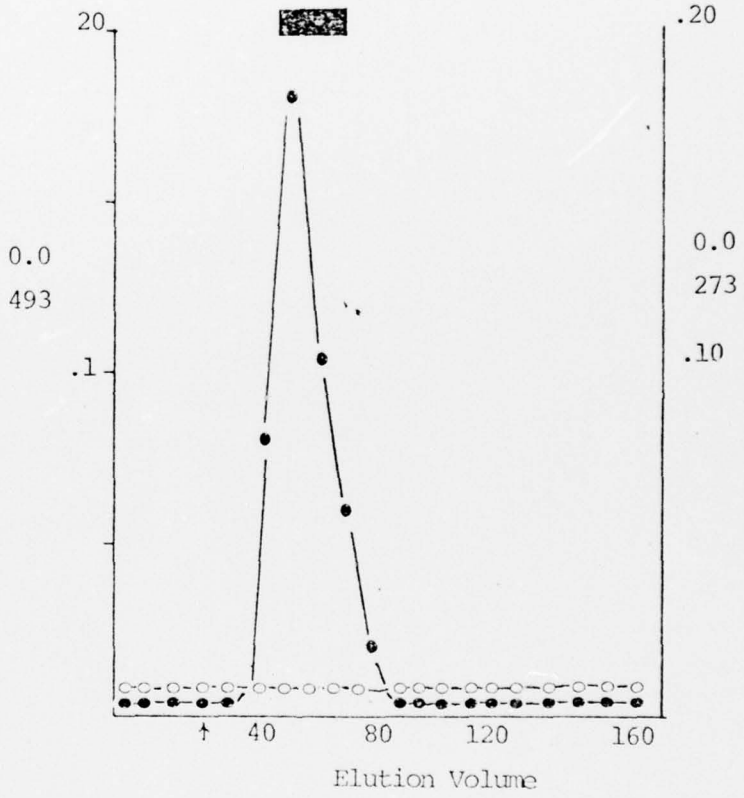


Figure 12. Sepharose 4B elution of antigen B-47. o-o represents 273 mμ absorption and ●-● represents 493 mμ absorption. Black bar indicates tubes containing antigen by immunodiffusion.

Figure 13

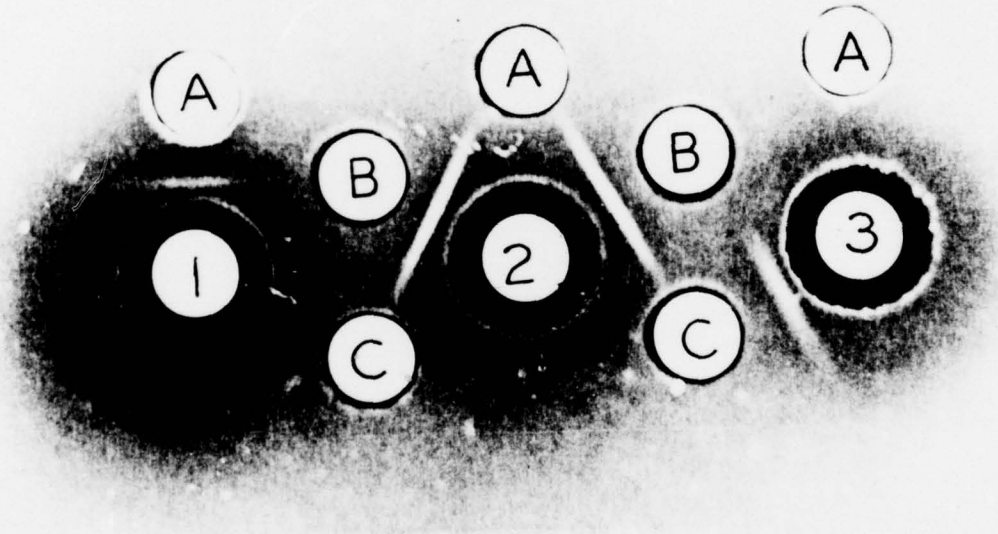


Figure 13 - Immunodiffusion study comparing 3 different group B non-capsular polysaccharide serotype antigens and absorbed antisera made to Gc_2 strain 1291 (Well 1), B-13 (Well 2), and B-51 (Well 3). Well A contains antigen B-47, Well B contains antigen B-13 and Well C contains antigen B-51.