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Auxotypes and Penicillin Susceptibilities of *Neisseria gonorrhoeae* Isolated from Patients with Gonorrhea Involving Two or More Sites

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A system of auxotyping described in 1973 is based on the differing nutritional requirement patterns of *Neisseria gonorrhoeae* strains. Our ongoing evaluation of the reliability of auxotyping has involved a study of the constancy of characteristics of gonococci isolated at one time from two or more sites of a given subject. The auxotypes and minimal inhibitory concentration (MIC) of penicillin G were determined for 181 isolates obtained from 84 patients with uncomplicated gonorrhea, for 16 isolates from 8 couples with uncomplicated gonorrhea, and for 21 isolates from 12 other patients, 9 with disseminated gonococcal infection and three consorts. The penicillin MIC served to distinguish between many members of auxotypes 1, 2, and 3, which are commonly involved in uncomplicated gonorrhea. Thus, for proline-requiring gonococci (auxotype 2) the MIC ranged from 0.01 to 1.2 IU of penicillin per ml. The profile of gonococcal responses to seven other antibacterial drugs provided useful additional information where the extent of phenotypic similarity was in doubt. In all but seven instances, the gonococci isolated from different sites of the same patient, or from a consort, had the same nutritional requirements and penicillin MIC. The gonococci isolated from one patient with disseminated gonococcal infection and from one of her two sexual contacts had nutritional requirements for arginine, hypoxanthine, uracil, and thiamine pyrophosphate, whereas the strain isolated from her second contact differed in having no requirement for thiamine pyrophosphate. The paired cervical and rectal isolates from one patient with uncomplicated gonorrhea differed only with respect to a requirement for hypoxanthine. Pairs of isolates from three patients differed slightly in degree of susceptibility to penicillin. In the remaining two instances, however, numerous differences between the isolates from the endocervix and the anal canal of a given patient indicated the presence of concomitant infections with different strains of *N. gonorrhoeae*.

Strains of *Neisseria gonorrhoeae* isolated from patients possess a variety of nutritional requirements, which provide a means of typing the isolates. Twenty auxotypes were discriminated based on the distinctive patterns of growth responses of 251 gonococcal strains inoculated on a standard set of 10 chemically defined agar media (2). The set comprised the complete neisseria defined agar medium, NEDA, and other media from which various components were separately omitted or a compound was substituted for others. L-Proline, L-arginine, L-ornithine, L-methionine, hypoxanthine, uracil, thiamine, and thiamine pyrophosphate were found to have differential value. Further investigations showed that some gonococcal isolates could be subdivided further by their responses to L-histidine, L-lysine, L-leucine, and L-glutamine (2, 8; B. W. Catlin, *Methods in Microbiology*, in press). Inclusion of requirements for these compounds in the typing schema has increased the number of auxotypes to 36.

Members of auxotype 1 are able to grow on each of the typing media, indicating that these bacteria possess functional enzymes for the biosynthesis of compounds which were omitted. Auxotype 1 accounted for one-fourth of an unselected group of 251 consecutive isolates from patients with uncomplicated gonorrhea who were examined in Milwaukee, Wisconsin, in 1972 (2). The remaining 187 strains were able to grow on the complete NEDA medium but were prevented from multiplying on one or more of the other auxotyping media because of various biosynthetic defects. Members of auxotype 2,

which are defined by a requirement for proline, comprised 33.8% of the isolates. Strains which required arginine but no other differential compound (auxotype 3) accounted for 17.5% of the 251 gonococci. Requirements for arginine, hypoxanthine, and uracil characterized 17.6% of the Milwaukee isolates. Such strains are of particular interest because they are incriminated frequently in disseminated gonococcal infections (1, 5, 8, 15).

The nutritional characteristics are relatively stable during cultivation of gonococci in various laboratory media (2, 15). Genetic transformation experiments verified that the various biosynthetic defects are hereditary and are repairable by deoxyribonucleic acid from suitable donor gonococci (4). Little is known, however, about the genetic variation of nutritional requirements of gonococci during multiplication in the tissues of persons with gonorrhea. The cervical, urethral, rectal, and oropharyngeal mucosa provide complex microenvironments in which significantly different selective forces might operate to diversify the gonococcal characteristics (B. W. Catlin, p. 453–466, *Microbiology-1976*, American Society for Microbiology, Washington, D. C., 1976). To obtain information about the constancy of nutritional requirements, we compared two or more isolates obtained from specimens taken at one time from different anatomical sites of a patient or from consorts. The auxotype and the minimal inhibitory concentration (MIC) of benzylpenicillin were determined for each isolate. The combination of these two determinations increased the proportion of gonococci that could be discriminated.

MATERIALS AND METHODS

Gonococci. Specimens from patients with uncomplicated gonorrhea were inoculated on Thayer-Martin medium (6), and cultures were incubated at 35°C in candle flame extinction jars. Colonies were selected on the basis of characteristic morphology, oxidase reaction, and microscopic appearance of the bacteria. Oxidase-positive, gram-negative diplococci were identified as *N. gonorrhoeae* by their production of acid from dextrose but not from maltose, sucrose, or lactose. An additional confirmatory characteristic was the nutritional requirement of gonococci for cysteine (or cystine), as shown by the absence of growth on the chemically defined medium from which cysteine and cystine were omitted and the presence of colonies on the complete defined medium (2). Gonococci isolated during the period from 1972 to 1976 were assigned Milwaukee Health Department code numbers and were stored at −60°C as dense suspensions of bacteria in 1-ml volumes of Trypticase soy broth (Baltimore Biological Laboratories, Cockeysville, Md.) supplemented with 20% glycerol.

Isolates from patients with disseminated gonococcal infections (DGI) were generously provided by Marjorie Bohnhoff (University of Chicago Hospitals and Clinics), by Alice Reyn (Statens Seruminstitut, Copenhagen, Denmark), by David Katzenstein (University Hospital, San Diego), and by Lynn Adams (Milwaukee County General Hospital). Strains from Copenhagen and from San Diego were lyophilized before being sent. Upon receipt, the gonococci were cultivated on GC medium base (Difco) containing 1% of the defined supplement described by White and Kellogg (18) (GCMBS). The identity of the isolates as *N. gonorrhoeae* was confirmed, and the bacteria were stored at −60°C.

Antibacterial drugs. Penicillin G potassium, lot number 795-2033 (potency, 1580 IU/mg), was kindly provided by J. C. Sylvester of Abbott Laboratories, North Chicago, Ill. Rifampin, lot number M1215K3886 of Rimactane (potency, 981 μg/mg), was generously supplied by E. A. Konopka, Ciba Pharmaceutical Co., Summit, N. J. Pfizer Inc., New York, N. Y., supplied dihydrostreptomycin, tetracycline, and chloramphenicol as preweighed and buffered preparations in a diagnostic testing kit. Tests of high concentrations (1, 000 μg/ml) of streptomycin sulfate used the medicinal product of Pfizer Inc. Oxacillin (Prostaphlin, Bristol Laboratories), erythromycin lactobionate (Abbott Laboratories), and sodium sulfadiazine (Lederle Laboratories) were medicinal products. A single lot of each drug was used throughout. Rifampin was dissolved in methyl sulfoxide (Aldrich Chemical Co., Milwaukee) at a concentration of 5,000 μg/ml, and further dilutions were made in sterile distilled water. A stock solution of 1 mg of penicillin G per ml of water was stored in 2-ml portions at −60°C. Other drugs were dissolved in water, and stock solutions usually were freshly prepared for addition to culture media.

Penicillin susceptibility assay. The assay medium was proteose peptone no. 3 agar (Difco) with 1% hemoglobin (Difco), 1% supplement C (Difco), and 0.3% dextrose prepared by a standard procedure (9). To portions of this molten medium, equilibrated to 56°C, were added appropriate dilutions of penicillin prepared from the freshly thawed (at 6°C) stock solution (protocol described by J. D. Thayer, J. E. Martin, Jr., and A. Lester, personal communication). Eleven penicillin concentrations and one penicillin-free control medium were used routinely. Expressed as international units per milliliter, these penicillin concentrations were: 1.0, 0.7, 0.5, 0.4, 0.3, 0.2, 0.1, 0.05, 0.01, 0.005, and 0.0025. In terms of micrograms of benzylpenicillin per milliliter, this range extended from 0.63 to 0.0016. For tests of gonococci resistant to 1.0 IU/ml, 10 additional concentrations of penicillin were used ranging from 1.1 to 2.0 IU/ml. Penicillin-containing medium was poured in 25-ml volumes into petri dishes (100 by 15 mm); excess surface moisture evaporated when the inverted plates were placed with covers offset in a 40°C incubator for 60 min. The medium was used, usually, soon after preparation. However, in tests of media stored at 6°C for 18 to 24 h and used after a warm-up period of 1 h at room temperature, the control bacteria displayed the standard susceptibilities, as others have reported (12).

In preparation for susceptibility tests, frozen cultures were thawed at 23°C and inoculated on antibiotic-free GC agar base (BBL) supplemented with 1% (wt/vol) hemoglobin and 1% (vol/vol) IsoVitaleX (BBL). Cultures were incubated at 35°C in a candle jar for 18 h. Cells were suspended in Trypticase soy broth (BBL), and the density was adjusted to 45% transmission of light at 530 nm in a Bausch and Lomb Spectronic 20 colorimeter (J. D. Thayer, J. E. Martin, Jr., and A. Lester, personal communication). A series of three 10-fold dilutions of the standardized suspension was prepared in Trypticase soy broth, and a 10^{-3} dilution was inoculated on the agar surfaces of the set of assay plates by either of two methods. During the early phase of this investigation, the agar was streaked manually with a 3-mm-calibrated platinum loop, which delivered 1×10^3 to 3×10^3 colony-forming units. However, most of the tests were performed using a Steers inocula-replicating device (17) (obtained from Melrose Machine Shop, Woodlyn, Pa.) that has 32 inoculating rods. The spots of inocula (which contained approximately 10^3 colony-forming units) were allowed to dry, and the plates were incubated at 35°C in candle flame extinction jars for 48 h. The MIC of benzylpenicillin was the lowest concentration of antibiotic that inhibited colony formation; a faint haze or an occasional single colony was disregarded. Two control cultures, included each time susceptibility tests were performed, were *N. gonorrhoeae* strain P-1 T-1 and *Sarcina lutea* (ATCC 9341) obtained from J. D. Thayer in 1967. The MIC of penicillin for strain P-1 T-1 was 0.2 IU/ml (0.15 IU/ml using a different dilution series; 12) and that for *S. lutea* was 0.005 IU/ml. These MIC values were obtained by both methods of inoculation of the penicillin media, and tests of 32 fresh gonococcal isolates by each method also yielded the same results.

Assays of resistance to other agents. GC medium base (Difco) with 1% of defined supplement (18) was used. The sterile molten agar was held at 56°C, and the solutions of each agent were added in the volumes required to give the following final concentrations (expressed as micrograms per milliliter): oxacillin, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0, 10.0, and 20.0; dihydrostreptomycin, 2.0, 5.0, 10.0, and 15.0; streptomycin, 1,000; rifampin, 0.02, 0.05, 0.1, 0.2, 0.5, 1.0, and 1.5; sulfadiazine, 2, 5, 10, 20, 35, 50, 100, and 150; tetracycline, 0.1, 0.2, 0.5, 1.0, 1.5, and 2.0; chloramphenicol, 0.1, 0.2, 0.5, 1.0, 1.5, 2.0, 3.0, and 4.0; erythromycin, 0.02, 0.05, 0.1, 0.2, 0.5, 1.0, 1.5, and 2.0. The media were stored at 4°C and were used not later than 4 to 5 days after preparation. The inocula were the same gonococcal suspensions used for auxotyping, as described below. *N. gonorrhoeae* strain P-1 T-1 was inoculated on each medium as a control. The plates were incubated at 36°C in an atmosphere of air plus 8% CO_2 for 48 h. These tests were performed at the Medical College of Wisconsin, whereas the penicillin susceptibility tests were conducted in the Milwaukee Health Department laboratory.

The highest concentration of the antibacterial agent that permitted growth of part of the gonococcal population (i.e., the formation of at least five colonies) was taken as the limit of resistance. This end point was normally one step below the MIC. Resistance expresses a definite characteristic of the population. Although the assigned value may be too low, it is free of the uncertainty of the MIC end point, which may be far too high depending on the intervals between the concentrations used in the assay. The apparent gonococcal resistances to various drugs were probably affected by the presence of starch (0.1%), which binds some compounds, dextrose (0.4%), which is metabolized to acid, and peptone, which may introduce *p*-aminobenzoic acid. However, reproducible results were obtainable.

Auxotyping. The chemically defined media were described earlier (2, 3), and the technical procedures have been given in greater detail recently (B. W. Catlin, in press). Soluble starch (0.1%, wt/vol; iodometric reagent grade, Merck) was added to all media. All of the amino acids used were L isomers. The set of auxotyping media included the complete NEDA, and NEDA with the omission of cysteine and cystine. The differential compounds singly omitted from NEDA included proline, arginine, methionine, hypoxanthine, and uracil. One medium lacked the mixture of vitamins and cofactors (−V medium; 2). Gonococci that failed to grow on −V medium were tested for growth on two additional media: −V medium supplemented with thiamine and −V medium supplemented with thiamine pyrophosphate. Gonococci that failed to grow on the arginine-free medium were examined additionally for ability to grow on NEDA that contained ornithine in place of arginine. Inasmuch as many arginine-requiring strains are able to grow when ornithine is substituted for arginine, the inability to use ornithine is a valuable differential characteristic. The above-described media were used in tests of all isolates. In addition, most of the gonococci (including all isolates enumerated in Tables 3 through 5) were examined on media with single omissions of histidine, lysine, and leucine. The strains listed in Table 5 were tested also for a possible requirement for glutamine.

The inocula were prepared from cultures on GCMBS agar incubated at 36°C in candle flame extinction jars for 16 to 18 h. Gonococci were suspended in buffered salts solution, and the density was adjusted (0.1 absorbance at 600 nm) as previously described (2). In the initial phase of this investigation the media were inoculated manually with the standardized suspensions (2). Later, the Steers apparatus was adopted, and, for this method of inoculation, the visibly turbid standard suspensions (which contained 1×10^8 to 3×10^8 colony-forming units/ml) were diluted with a calibrated 0.01-ml platinum loop, transferring a full loopful to 0.5 ml of the buffered salts solution. The inocula delivered by the Steers rods (0.0025 ml, estimated volume; 5) thus contained between 10^3 and 10^4 colony-forming units. The auxotyping media were inoculated first, followed in order by a control plate of GCMBS, the various antibiotic-containing media, and finally a second plate of GCMBS. When the inocula were dry, the media were incubated at 36°C in air plus 8% CO_2 for 48 h (2).

The reference strains of *N. gonorrhoeae* described earlier (2) were used to monitor the growth-promot-

ing qualities of NEDA and to ascertain that the growth responses obtained on the differential media were typical. Similar amounts of growth of a given strain were normally found on the complete NEDA medium and on GCMBS. The requirement for a differential compound was indicated by the absence of macroscopic growth on a medium lacking that compound; a haze of microcolonies or the presence of a single macrocolony was disregarded. The distinctive patterns of nutritional requirements that define the previously recognized auxotypes were summarized earlier (2; B. W. Catlin, in press). The auxotypes represented by the strains of *N. gonorrhoeae* described in this communication are listed in Table 1.

RESULTS

Simultaneously infected sites: uncomplicated gonorrhea. When a person develops gonorrhea after one sexual episode, the gonococci that may be isolated subsequently from various exposed sites (urogenital tract, and canal, and oropharynx) should possess the same nutritional requirements and antibiotic susceptibilities, provided the infecting population does not undergo genetic change during multiplication in the different tissues. Tests of the *N. gonorrhoeae* isolates from five women supported this expectation; the auxotype and MIC of penicillin G were identical for the pairs of gonococci isolated the same day from the endocervix and anal canal of a given patient (2). We have extended this study by examining gonococci isolated from specimens taken at one time from two or three anatomical sites of each of 84 patients with uncomplicated gonorrhea.

The same auxotype and penicillin MIC were found for the two or three isolates from different specimens from each of 78 patients (Table 2). The several indistinguishable isolates from a given patient are referred to here as one strain. Although the majority of the strains were classified in auxotypes 1 (27%), 2 (36%), or 3 (24%), it was possible to differentiate between many members of each auxotype on the basis of differing susceptibilities to penicillin. The 10 remaining strains listed in Table 2 displayed a narrower range of susceptibilities but were grouped into eight auxotypes. The penicillin MIC values obtained with some of the matched isolates from these 78 patients differed by one dilution in initial tests, but further tests established an identity of the MIC values.

Pairs of isolates from three of the 84 patients differed as to auxotype. Three other pairs had small but reproducible differences of penicillin MIC, verified in up to seven tests. Isolates from patient MM (Table 3) differed in both respects. To obtain additional information that might help to establish whether or not the two isolates from each of these six patients were genuinely different strains, tests were made of the degrees of resistance to other antibacterial drugs (Table 3). For comparison, Table 4 presents the results of similar tests performed on isolates from 11 of the 78 patients (Table 2) who were considered to have the same strain at all sampled sites; these sets of isolates were selected on the basis of their differing susceptibilities to penicillin.

The data in Table 3 strongly suggest that the cervical and rectal isolates from patient MM represent two distinct infections. Isolate 667 differs from 661 by the absence of requirements for proline and arginine, by small but repeatable differences of resistance to penicillin, chloramphenicol, and rifampin, and by gross differ-

TABLE 1. *Patterns of requirements for differential compounds that identify the gonococcal auxotypes found in this study*

Auxotype	Differential compound required for growth
1	None
2	Proline
3	Arginine
4	Methionine
5	Thiamine
7	Proline, arginine
9	Proline, hypoxanthine
14	Arginine, hypoxanthine, uracil
16	Arginine,[a] hypoxanthine, uracil
19	Arginine, hypoxanthine, uracil, thiamine pyrophosphate
21	Proline, thiamine pyrophosphate
23	Proline, thiamine
24	Arginine, thiamine
30	Arginine, hypoxanthine, uracil, leucine
34	Proline, arginine,[a] hypoxanthine, uracil, methionine, leucine
35	Proline, arginine, hypoxanthine, uracil, thiamine pyrophosphate
36	Arginine, hypoxanthine, uracil, glutamine

[a] Requirement for arginine is not satisfied by ornithine.

TABLE 2. *Characteristics of various N. gonorrhoeae strains (162 isolates) obtained from 78 patients infected at two or three anatomical sites with a single strain*

Patients		No. of patients	Auxotype	No. of strains[a] susceptible to indicated MIC (penicillin G, U/ml)									
Sites[b]	Sex			0.01	0.05	0.1	0.2	0.3	0.4	0.5	0.7	1.0	1.2
OP + U	M	1	1						1				
OP + R	M	2	1			1	1						
OP + C	F	3	1	2		1							
C + R	F	14	1	1	3	4[c]	1	3	1			1	
U + R	M	1	1				1						
OP + C	F	2	2				1		1				
OP + C + R	F	4	2				1					2[d]	1[c]
C + R	F	22	2	2	3	3	3[c]	3	2	2[e]	1	3[c, e]	
OP + U	M	1	3					1					
OP + R	M	1	3					1					
OP + C	F	2	3		1						1		
OP + C + R	F	2	3				1	1					
C + R	F	13	3		2	3		1	1	4	1	1	
OP + C	F	1	4			1							
C + R	F	2	5		2[c]								
C + R	F	2	14	2									
C + R	F	1	16	1									
C + R	F	1	21		1								
C + R	F	1	23	1									
OP + C	F	1	24				1						
OP + C	F	1	34	1									

[a] The term "strain" is applied to two or more indistinguishable gonococcal isolates cultured from different specimens taken at one time from a given patient.

[b] Abbreviations: OP, oropharynx, U, urethra, R, rectum; C, cervix or, for two patients, vagina.

[c] Strains isolated from one or two of these patients were characterized in Table 4 of reference 2.

[d] The same gonococcal strain was isolated from OP and C specimens taken after treatment of one patient as test-of-cure.

[e] The same gonococcal strain was isolated from C and R specimens taken at a later time from one of these patients.

ences of resistance to oxacillin, erythromycin, and sulfadiazine. The low resistance of 667 to oxacillin (0.1 μg/ml) is unusual for a strain characterized by a MIC of 0.1 U of penicillin per ml (compare 667 to isolates 987 and 847, Table 4). Also, the differing nutritional requirements of isolates 762 (Arg^-) and 741 (Pro^-) together with the large differences of resistance to streptomycin and sulfadiazine and the smaller differences of response to chloramphenicol and erythromycin indicate that these two isolates cultured from two specimens taken simultaneously from patient SG (Table 3) are different strains. On the other hand, the similarities of response to the various drugs other than penicillin suggest that the penicillin MIC differences between the pairs of isolates from patients BG, JB, and DY (Table 3), although reproducible, were not significant. It is likely that the infection of patient SP was initiated by gonococci with requirements for proline and hypoxanthine (Pro^- Hyx^-) and that these requirements were retained during multiplication in the anal canal from which 714 was isolated. However, the requirement for hypoxanthine was lost by isolate 703 either before or after the specimen was obtained from the cervix. Two other Pro^- Hyx^- strains are known to have changed during laboratory manipulations, with the result that hypoxanthine is no longer an absolute nutritional requirement although it strongly enhances their growth; they retained the requirement for proline (unpublished observations).

Multiple sites: DGI. Relatively few multiply auxotrophic gonococci were found among these isolates from patients with uncomplicated gonorrhea. Therefore, to evaluate the genetic stability in vivo of additional strains likely to possess complex nutritional requirements (5), we compared the isolates from two or more sites from seven patients with DGI and also the gonococci obtained from two additional patients with DGI and from their recent sexual partners. The auxotype and penicillin MIC were the same for the isolates from different sites of a given patient and, also, for the gonococci isolated from patient 8 and from his consort (Table 5). The isolates from patient 10 and her two consorts were alike in their antibiotic resistance profiles and the requirements for arginine, hypoxanthine, and uracil, but differed with respect to the requirement for thiamine pyrophosphate. If the infection of patient 11

TABLE 3. *Auxotypes and responses to antibacterial drugs:*[a] *N. gonorrhoeae isolated from cervical (C) and rectal (R) specimens taken at one time from each of six patients (not represented in Table 2)*

Patient	Specimen	Code no.	Auxotype	MIC of penicillin (U/ml)	Highest concentration (μg/ml) of drug permitting growth						
					Oxacillin	Streptomycin	Tetracycline	Chloramphenicol	Erythromycin	Rifampin	Sulfadiazine
SP	C	703	2	0.7	10.0	≥1,000	0.5	0.5	0.2	0.1	5.0
SP	R	714	9	0.7	10.0	≥1,000	0.5	0.5	0.2	0.2	5.0
MM	C	667	1	0.1	0.1	≥1,000	0.2	1.0	0.05	0.02	50.0
MM	R	661	7	0.2	1.0	≥1,000	0.2	1.5	0.5	0.05	10.0
SG	C	762	3	0.2	2.0	10	0.1	0.5	0.5	0.1	5.0
SG	R	741	2	0.2	2.0	≥1,000	0.1	0.2	0.2	0.1	50.0
BG	C	820	1	0.7	5.0	≥1,000	0.5	1.5	0.5	0.1	20.0
BG	R	819	1	0.5	5.0	≥1,000	0.5	1.5	0.5	0.1	20.0
JB	C	665	2	1.2	10.0	≥1,000	0.5	0.2	0.2	0.1	10.0
JB	R	666	2	1.0	10.0	≥1,000	0.5	0.2	0.2	0.1	10.0
DY	C	851	3	0.2	2.0	≥1,000	0.2	0.5	1.5	0.1	5.0
DY	R	830	3	0.4	2.0	≥1,000	0.2	0.5	1.5	0.1	5.0

[a] Responses to penicillin expressed as MIC (units per milliliter) of penicillin G were assayed by a method different from that used to determine the degrees of resistance to the other drugs (see Materials and Methods).

TABLE 4. *Auxotypes and responses to antibacterial drugs.[a] N gonorrhoeae cultured at one time from two or three anatomical sites of 11 patients (one line for each patient; patients selected from those of Table 2)*

Sites[b]	Isolate code no.	Auxotype	MIC of penicillin (U/ml)	Highest concentration (μg/ml) of drug permitting growth						
				Oxacillin	Streptomycin	Tetracycline	Chloramphenicol	Erythromycin	Rifampin	Sulfadiazine
OP + C	987, 986	1	0.01	0.1	5	0.1	0.2	0.1	0.02	10
C + R	698, 689	1	0.3	2.0	≥1,000	0.2	1.5	0.2	0.05	20
C + R	756, 763	1	1.0	5.0	≥1,000	0.5	3.0	1.0	0.2	≥150
C + R	847, 849	2	0.01	0.1	5	0.1	0.2	0.2	0.05	20
C + R	844, 845	2	0.1	1.0	10	0.1	0.2	0.2	0.1	2
V + R	646, 647	2	0.4	2.0	5	0.2	0.2	0.2	0.1	50
OP + C + R	874, 868, 870	2	1.2	5.0	≥1,000	0.5	0.2	0.2	0.1	5
OP + C + R	652, 648, 653	3	0.2	2.0	5	0.2	0.5	1.0	0.1	5
C + R	684, 687	3	0.5	5.0	≥1,000	0.5	1.0	0.5	0.2	35
C + R	712, 664	3	1.0	5.0	≥1,000	0.2	1.0	0.5	0.2	20
OP + C	996, 997	34	0.01	0.2	5	0.1	0.2	0.2	0.1	50

[a] Responses to penicillin expressed as MIC (units per milliliter) of penicillin G were assayed by a method different from that used to determine the degrees of resistance to the other drugs (see Materials and Methods).

[b] OP, Oropharynx; C, cervix; V, vagina; R, rectum.

was acquired from patient 10, then the nutritional requirement for thiamine pyrophosphate, which characterizes strain 160169, was lost by strain 163993. This change might occur either during multiplication of the gonococcal population in vivo or subsequently during the numerous subcultures in the laboratory media used for isolation, purification, and growth of the bacteria before or after lyophilization.

The antibiotic resistance profiles were remarkably similar for all of the gonococci whose nutritional requirements included arginine, hypoxanthine, and uracil (AHU strains). All were susceptible to low concentrations of penicillin G, as other investigators have reported (5, 8, 15). Furthermore, the 17 AHU isolates characterized in Table 5, together with isolates 996 and 997 (Table 4), displayed relatively similar resistance profiles: low resistance to oxacillin (0.1 or 0.2 μg/ml), streptomycin (2 or 5 μg/ml), tetracycline (0.1 μg/ml), chloramphenicol (0.2 μg/ml), and erythromycin (0.1 or 0.2 μg/ml). The degrees of resistance of rifampin ranged from 0.02 to 0.1 μg/ml. Sulfadiazine resistance, which is often not correlated with resistance to antibiotics (e.g., Table 4), ranged from 5 to 20 μg of sulfadiazine per ml for the AHU strains of Table 5. The auxotype 2 isolates from the four specimens collected simultaneously from patient 7 (Table 5) displayed profiles of resistance to penicillin and to oxacillin (2.0 μg/ml), tetracycline (0.2 μg/ml), chloramphenicol (1.0 μg/ml), erythromycin (0.5 μg/ml), and sulfadiazine (100 μg/ml) that were identical to one another but were elevated in comparison to the other DGI isolates.

Urogenital isolates from consorts. Gonococci were isolated from both members of eight couples who attended the Social Hygiene Clinic together. In each instance, the auxotypes and penicillin susceptibilities were the same for the isolates from the endocervix of the woman and the urethra of her male sexual partner. There were two pairs of auxotype 1 isolates with MIC values of 0.1 and 0.2 U of penicillin per ml, respectively. Of the auxotype 2 isolates, one pair had an MIC of 0.01 and the other pair an MIC of 0.1. Three pairs of auxotype 3 gonococci were represented, and all six isolates had the same MIC (0.7 U of penicillin per ml). Specimens from couple eight yielded auxotype 7 gonococci, and the MIC of each was 0.1 U of penicillin per ml.

DISCUSSION

The endocervix, anal canal, urethra, and oropharynx each provide different microenvironments for the gonococci because of their differ-

TABLE 5. *Auxotype and susceptibility to penicillin G of isolates from two or more specimens from patients with DGI and consorts*

Patient	Sex	Sites[a]	City	Laboratory no.	Auxotype	Penicillin MIC (U/ml)
1	M	S + B	Milwaukee	MCGH 7/76, 8/76	14	0.05
2	F	V + S	Milwaukee	MCGH 11/76, 10/76	14	0.05
3	F	C + B	San Diego	BC, BB	36	0.01
4	F	C + Sk	Chicago	415, 412	35	0.05
5	F	C + S	Chicago	450, 449	30	0.01
6	F	C + B	Chicago	745, 746	16	0.01
7	F	C + R + S + B	Chicago	S753, C, R, J, B	2	0.4
8	M	B	Chicago	S826	14	0.05
9[b]	F	C	Chicago	A827	14	0.05
10[c]	F	Genital	Copenhagen	SS 160169	19	0.05
11[d]	M	U	Copenhagen	SS 163993	14	0.05
12[d]	M	U	Copenhagen	SS 164014	19	0.05

[a] Abbreviations: S, synovial effusion; B, blood; V, vagina; C, cervix; Sk, skin lesion; R, rectum; U, urethra.

[b] Asymptomatic sexual contact of patient 8.

[c] Patient was hospitalized for treatment of DGI; patient 10 also was treated for DGI 4 months earlier when gonococci were isolated from genital and cerebrospinal fluid specimens.

[d] Sexual contact of patient 10.

ing kinds of epithelial cells and fluids that bathe these tissues and their distinctive, normal microbial floras. When an initially homogeneous population of gonococci is introduced into a new host and becomes dispersed into several microenvironments, different selective factors may operate to increase the diversity of *N. gonorrhoeae* (B. W. Catlin, p. 453–466, *Microbiology-1976*, American Society for Microbiology, Washington, D. C., 1976). The finding that gonococci possess numerous and varied nutritional requirements raised the question as to whether, during the progress of infection, the bacterial populations frequently undergo genetic changes affecting these requirements.

The results of the multisite isolate study described here indicate that the nutritional requirements are relatively stable during multiplication of the gonococci in the different tissues of patients. A difference between the auxotypes of two otherwise similar isolates from the same patient was found only once (patient SP, Table 3); this involved a loss of the strict requirement for hypoxanthine by a strain that retained its requirement for proline. In 81 instances the same nutritional requirements characterized the strains isolated at one time from two or three different sites of any one patient with uncomplicated gonorrhea (Tables 2 and 3). The analyses of gonococci from patients with disseminated infections and from their consorts showed a correspondence between the two or more isolates from related sources with respect to the requirements for all compounds except thiamine pyrophosphate (patient 11, Table 5). Eleven of these patients were infected with gonococci characterized by multiple requirements that included arginine, hypoxanthine, and uracil (AHU strains). The hypoxanthine requirement of the AHU strains was stable during multiplication in patients. Furthermore, we have auxotyped more than 90 different AHU strains, many of which have been subcultured repeatedly on laboratory media, and have never detected a spontaneous change involving their requirements for arginine, hypoxanthine, or uracil (2; unpublished observations).

Knapp and Holmes (5) also studied gonococci recovered from more than one site during one visit from 13 patients; in each case, isolates from multiple sites were of identical auxotype. Similarly, five patients who were examined more than once before treatment yielded gonococci with corresponding auxotypes (5). Another study established that one patient was successively infected 10 times with a strain of *N. gonorrhoeae* that required arginine, hypoxanthine, uracil, and leucine. The source of reinfection was eradicated only after auxotyping had successfully distinguished between a "false" and a "true" conjugal partner (8).

The results in Table 2 show that in many instances the MIC of penicillin serves as a means of distinguishing between members of each of the three commonest auxotypes. The reliability of this discrimination was increased by obtaining profiles of resistances to additional antibacterial drugs. Differences between pairs of isolates with respect to both the auxo-

type and the resistance profile showed that in two instances a patient had concomitant infections with different gonococcal strains (patients MM and SG, Table 3). Other investigators also have used differing gonococcal susceptibilities to a number of drugs to differentiate between treatment failure and reinfection (7, 10, 16) or between "false" and "true" conjugal partners (14). Furthermore, for several years workers at the State Serum Institute in Copenhagen, Denmark, have compared the resistances to penicillin, tetracycline, and streptomycin of gonococci isolated from specimens taken simultaneously from the throat and from one or more other sites. Differences of the IC_{50} value (50% inhibitory concentration; 12) greater than 4-fold for penicillin and greater than 2.8-fold for tetracycline indicated nonidentity of the isolates. Such differences combined with streptomycin susceptibility of one member of the pair and streptomycin resistance of the other led to the conclusion that patients may harbor at least two different gonococcal strains at one time (A. Reyn, personal communication). Strains of gonococci isolated in 1975 from two simultaneous specimens from a male subject were received through the kindness of A. Reyn. The isolates from the urethra (strain 156534, auxotype 1, streptomycin susceptibile) and the throat (strain 156536, auxotype 2, streptomycin resistant) exhibited 10-fold or greater differences of resistance to penicillin, oxacillin, chloramphenicol, erythromycin, and vancomycin (data not shown); two- to fourfold differences were found for resistances to tetracycline, rifampin, and bacitracin.

The resistance profile of strain 667, the auxotype 1 isolate from patient MM (Table 3), was unusual in its low resistances to oxacillin, erythromycin, and rifampin combined with a significantly increased resistance to penicillin G. More commonly, members of auxotypes 1 and 3 that were moderately resistant to penicillin G also displayed increased resistances to oxacillin, erythromycin, and some other drugs. Such correlated increases of resistances to penicillin, streptomycin, tetracycline, and erythromycin have been documented by other investigators (11, 13). However, the proline-requiring strains (auxotype 2, Tables 3 and 4) were exceptional in that an increased resistance to penicillin was not associated with an increase of resistance to erythromycin. All of the AHU strains (Tables 4 and 5) were susceptible to penicillin, confirming the findings of other studies (5, 8, 15). Furthermore, all AHU strains were susceptible also to various other antibacterial drugs, except sulfadiazine. These susceptibilities were not unique to AHU strains, however, but were displayed by some gonococci with simpler nutritional requirements (Table 4).

Although the nutritional requirements of gonococci are remarkably stable both in vivo and in vitro, it is apparent that gonococci classified in different auxotypes differ in their ability to multiply in different microenvironments. DGI are caused more often by AHU strains than by members of auxotypes 1, 2, or 3 (1, 5, 8, 15). On the other hand, there appears to be an association between greater biosynthetic competence and the ability of gonococci to persist in the oropharynx (Catlin and Pace, Br. J. Vener. Dis., in press). This association is reflected in the data of Table 2, which show that oropharyngeal isolates were obtained from 27% of the 78 patients with multisite infections; 86% of these 21 patients were infected with strains classified in auxotypes 1, 2, and 3. This is a significantly higher percentage than was found in two surveys of consecutive isolates primarily from cervical specimens. One group of 251 isolates collected in 1972 contained only 77% of gonococci representing auxotypes 1, 2, and 3 (2). A study of isolates obtained 3 years later from 97 patients attending the same clinic showed that 73% of the gonococci were members of auxotypes 1, 2, and 3 (manuscript in preparation). AHU strains comprised 18% of both groups of consecutive isolates. However, AHU strains were responsible for infections of only 4 (5%) of the selected group of 78 patients listed in Table 2 and none of those of Table 3. These results suggest that the biosynthetically less competent AHU strains are less adapted for survival and growth in the oropharynx than are the nutritionally more independent members of auxotypes 1, 2, and 3. Accordingly, one may infer that the percentage of AHU strains actually recovered from the oropharynx was disproportionately lower than the percentage initially introduced. Recent evidence indicates that a high proportion of the AHU strains responsible for DGI are resistant to the bactericidal activities of human serum (1, 15). This attribute would be less useful in promoting gonococcal survival in the oropharynx than in the blood stream, for example. Characteristics of the less fastidious auxotype 1, 2, and 3 strains that may favor persistence in the oropharynx are obscure at present.

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LITERATURE CITED

1. **Brooks, G. F., K. S. Israel, and B. H. Petersen.** 1976. Bactericidal and opsonic activity against *Neisseria gonorrhoeae* in sera from patients with disseminated gonococcal infection. J. Infect. Dis. 134:450–462.
2. **Carifo, K., and B. W. Catlin.** 1973. *Neisseria gonorrhoeae* auxotyping: differentiation of clinical isolates based on growth responses on chemically defined media. Appl. Microbiol. 26:223–230.
3. **Catlin, B. W.** 1973. Nutritional profiles of *Neisseria gonorrhoeae, Neisseria meningitidis,* and *Neisseria lactamica* in chemically defined media and the use of growth requirements for gonococcal typing. J. Infect. Dis. 128:178–194.
4. **Catlin, B. W.** 1974. Genetic transformation of biosynthetically defective *Neisseria gonorrhoeae* clinical isolates. J. Bacteriol. 120:203–209.
5. **Knapp, J. S., and K. K. Holmes.** 1975. Disseminated gonococcal infections caused by *Neisseria gonorrhoeae* with unique nutritional requirements. J. Infect. Dis. 132:204–208.
6. **Martin, J. E., Jr., T. E. Billings, J. F. Hackney, and J. D. Thayer.** 1967. Primary isolation of *N. gonorrhoeae* with a new commercial medium. Public Health Rep. 82:361–363.
7. **Masterson, G., and C. B. S. Schofield.** 1972. Doxycycline HCl (Vibramycin) as a single dose oral treatment of gonococcal and nonspecific urethritis in men. Br. J. Vener. Dis. 48:121–125.
8. **Morello, J. A., S. A. Lerner, and M. Bohnhoff.** 1976. Characteristics of atypical *Neisseria gonorrhoeae* from disseminated and localized infections. Infect. Immun. 13:1510–1516.
9. **Public Health Service Publication no. 499.** 1963. Gonococcus—procedures for isolation and identification, p. 1–39. United States Government Printing Office, Washington, D. C.
10. **Reyn, A., and M. W. Bentzon.** 1963. Sensitivity to antibiotics of gonococcal strains isolated by repeated culturing from the same patient. Acta Derm. Venereol. 43:394–398.
11. **Reyn, A., and M. W. Bentzon.** 1968. A study of the relationships between the sensitivities of *Neisseria gonorrhoeae* to sodium penicillin G, four semi-synthetic penicillins, spiramycin, and fusidic acid. Br. J. Vener. Dis. 44:140–150.
12. **Reyn, A., M. W. Bentzon, J. D. Thayer, and A. E. Wilkinson.** 1965. Results of comparative experiments using different methods for determining the sensitivity of *Neisseria gonorrhoeae* to penicillin G. Bull. W.H.O. 32:477–502.
13. **Reyn, A., B. Korner, and M. W. Bentzon.** 1958. Effects of penicillin, streptomycin, and tetracycline on *N. gonorrhoeae* isolated in 1944 and in 1957. Br. J. Vener. Dis. 34:227–239.
14. **Schmidt, H., and S. O. Larsen.** 1962. Comparison of the *in vitro* sensitivity of gonococcal strains isolated from patients and from their contacts. Acta Derm. Venereol. 42:294–304.
15. **Schoolnik, G. K., T. M. Buchanan, and K. K. Holmes.** 1976. Gonococci causing disseminated gonococcal infection are resistant to the bactericidal action of normal human sera. J. Clin. Invest. 58:1163–1173.
16. **Silver, P. S., and W. M. Darling.** 1971. Penicillin-insensitive gonococci in the Bolton area. Preponderance in young women and immigrants. Br. J. Vener. Dis. 47:367–372.
17. **Steers, E., E. L. Foltz, and B. S. Graves.** 1959. Inocula replicating apparatus for routine testing of bacterial susceptibility to antibiotics. Antibiot. Chemother. (1954–68) 9:307–311.
18. **White, L. A., and D. S. Kellogg, Jr.** 1965. *Neisseria gonorrhoeae* identification in direct smears by a fluorescent antibody-counterstain method. Appl. Microbiol. 13:171–174.