Auxotypes of *Neisseria gonorrhoeae* isolated from localized and disseminated infections in Montreal

PIERRE L. TURGEON,* MD, M SC, FRCP[C]; M. JOLIVET GRANGER,† MD

A survey recently made in the United States on the regional distribution of auxotypes of *Neisseria gonorrhoeae* suggested that isolates from different geographic areas often differ in auxotype. A subsequent auxotyping study in Montreal of 901 isolates of *N. gonorrhoeae*, 15 from patients with disseminated gonococcal infection, proved interesting in many regards.

Gonococcal genetic medium, modified by the addition of other amino acids, was used. Most (93%) of the strains isolated from patients with localized infection belonged to one of the following three phenotypes: arginine-, hypoxanthine- and uracil-dependent (44%); prototrophic (33%); and proline-dependent (16%). Of the 15 strains responsible for disseminated infection 14 required arginine, hypoxanthine and uracil for growth.

Une étude faite récemment aux États-Unis portant sur la distribution des auxotypes de *Neisseria gonorrhoeae* révélait que les souches isolées n'appartenaient pas nécessairement aux mêmes auxotypes d'une région à l'autre. Une étude similaire à Montréal de 901 souches de *N. gonorrhoeae*, dont 15 avaient été isolées de patients souffrant de gonococcémie, s'avéra des plus intéressantes.

Le milieu génétique gonococcique, modifié par l'addition d'autres acides aminés, a été utilisé. La plupart (93%) des souches isolées de patients souffrant de gonorrhée localisée appartenaient à l'un des trois phénotypes suivants: arginine, hypoxanthine et uracile dépendant (44%); prototrophique (33%); et proline dépendant (16%). Des 15 souches provenant de cas de gonococcémie 14 exigeaient pour croître la présence d'arginine, d'hypoxanthine et d'uracile.

In the early 1970s Catlin recognized that the nutritional requirements of gonococcal isolates vary. She accordingly developed a chemically defined medium, called *Neisseria*-defined agar, that permitted the classification of gonococci into several auxotypes on the basis of their requirements for proline, arginine, methionine, histidine, lysine, leucine, hypoxanthine, uracil and vitamins.[1-4] Shortly thereafter LaScolea and Young[5] developed a somewhat less complex defined medium, called gonococcal genetic medium, by means of which strains could be divided into eight major and minor phenotypes according to their requirements for proline, arginine, serine, isoleucine, cystine and cysteine.

The hereditary basis of these auxotypes and their stability in vitro were verified by transformation tests involving deoxyribonucleic acid (DNA) and *Neisseria*-defined agar.[6] The results suggested that mutations responsible for the biosynthetic defects in the elaboration of a given amino acid by a gonococcal strain affect genes. Once these properties were confirmed, auxotyping could be considered of great value for epidemiologic studies of gonorrhea.

Several epidemiologic studies of the prevalence of *N. gonorrhoeae* auxotypes have been conducted, mostly in the United States. Four major phenotypes have been recognized: prototrophic (not requiring any particular single substance); proline-dependent; arginine-dependent; and arginine-, hypoxanthine- and uracil-dependent. Substantial regional differences were found in the proportion of strains requiring arginine, hypoxanthine and uracil, and of other auxotypes: in most cities in which such a study was conducted 8% to 22% of the gonococci isolated required these three amino acids; however, the proportion was more than 50% in Seattle and Des Moines (Iowa), whereas in the Philippines no such strain was isolated (Table I).[7-9] These variations may be related to variations in the proportion of cases of disseminated gonococcal infection in these regions. This clinical syndrome is rarely encountered in the Philippines, whereas it is found in 1% to 3% of all patients with gonococcal infection in Seattle who present themselves for treatment. We therefore decided to study the distribution of auxotypes of *N. gonorrhoeae* responsible for localized and disseminated infections in Montreal.

Materials and methods

Strains

Auxotyping was done for strains of *N. gonorrhoeae* isolated during the 34-month period March 1977 to December 1979 from 825 patients with localized infection and 15 patients with disseminated infection. The diagnosis of disseminated infection was considered if gonococci were cultured from synovial fluid, skin lesions or blood, or if suggestive skin lesions or arthritis was present and gonococci were recovered simultaneously from the anogenital or pharyngeal area. Each strain showing *Neisseria*-like properties on Thayer–Martin medium was subcultured on gonococcal medium base (Difco, Detroit) to which a commercially available supplement (IsoVitaleX, Baltimore Biological Laboratories, Cockeysville, Maryland) had been added and was further identified by sugar fermentation in a modified rapid carbohydrate utilization test.[10] All strains were stored at −70°C as dense suspensions of bacteria in 3 ml of trypticase soy broth supplemented with glycerol (15% by volume).

Media

Gonococcal genetic medium was prepared as de-

From the department of microbiology and immunology, University of Montreal, and le service de microbiologie, hôpital Saint-Luc, Montreal

*Professor of microbiology, University of Montreal
†Resident in microbiology, hôpital Saint-Luc

Reprint requests to: Dr Pierre L. Turgeon, Service de microbiologie, Hôpital Saint-Luc, 1058, rue Saint-Denis, Montréal, PQ H2X 3J4

scribed by LaScolea and Young.[5] Because some strains are stimulated by L-valine, L-histidine, L-leucine and L-lysine the medium was modified to include these four amino acids.[11] Furthermore, the sodium bicarbonate concentration was increased from 5% to 10%, since the higher concentration enhances growth (A.T. Hendry: personal communication, 1977). The set of media included complete gonococcal genetic medium, this medium without a given amino acid (proline, arginine, methionine or leucine) or nitrogen base (hypoxanthine or uracil) and this medium without cystine and cysteine, which differentiates meningococci from gonococci. Arginine-free gonococcal genetic medium supplemented with ornithine (which is obtained from arginine by splitting off urea) was added when it was noticed that many strains required arginine for growth. The media were dispensed into Petri dishes, individually sealed with paraffin paper and kept at 4°C for up to 2 months.

Auxotyping procedure

The frozen suspensions of bacteria were thawed at room temperature and inoculated on gonococcal medium base at 37°C in candle extinction jars. After 18 to 24 hours the gonococci were suspended in 0.5 ml of buffer salt solution (McFarland barium sulfate standard no. 1)[2] to a concentration of approximately 10^8 colony-forming units per millilitre. The Steers replicating apparatus[12] was then used to deposit 10^4 to 10^5 colony-forming units per millilitre on each auxotyping medium.

The requirement for an amino acid was indicated by an absence of macroscopic growth, a haze of microcolonies or two or fewer macrocolonies on a medium lacking that amino acid after incubation at 37°C in carbon dioxide for 48 hours.

To monitor the growth-promoting qualities of gonococcal genetic medium and to ascertain that the growth responses were typical on the differential media, we included reference strains 27628, 27630, 27631, 27632 and 27633 from the American Type Culture Collections each time auxotyping was performed.

Results

Localized infections

From the 825 patients with localized anogenital or pharyngeal infection 886 strains of *N. gonorrhoeae* were isolated and auxotyped. A second infection occurred in 59 of the patients during the survey period. Two patients were simultaneously infected by two auxotypes.

Of the 886 strains 97% were prototrophic or required either proline or arginine, hypoxanthine and uracil (Table II); 44% failed to grow in the absence of arginine, hypoxanthine and uracil, and some of these required other nutrients, such as leucine.

In 16 of the 23 patients who had a second infection within 6 weeks of the first, the auxotypes of the strains responsible for the two infections were identical.

Disseminated infections

Fifteen patients (12 women and 3 men) were admitted to hospital during the study period with a clinical syndrome consistent with disseminated gonococcal infection; only 2 of the patients were related to each other. *N. gonorrhoeae* was isolated from blood specimens in two cases and from swabs of the ano-

Table I—Regional differences in distribution of *Neisseria gonorrhoeae* auxotypes[7–9]

Country or city, year (and no. of isolates)	Auxotype (% of all isolates)				
	Prototrophic	Proline-dependent	Arginine-dependent	Arginine-, hypoxanthine- and uracil-dependent	Others
Philippines and Taiwan, 1975 (91)	59.3	38.5	0	0	2.2
Seattle, 1975 (114)	18.4	15.7	6.1	56.1	3.7
Denver, 1978 (49)	28.5	34.6	8.1	22.4	6.4
Des Moines, Iowa, 1975 (49)	12.2	16.2	2.3	57.1	12.2
Chicago, 1975 (216)	21.7	23.1	13.8	32.4	9.0
Boston, 1978 (50)	38	34	20	8	0
Miami, 1978 (49)	40.7	34.6	12.2	10.2	2.3

Table II—Distribution of *N. gonorrhoeae* auxotypes isolated from patients in Montreal

Type of infection	No. of patients	No. of strains	Auxotype; no. (and %) of isolates				
			Prototrophic	Proline-dependent	Arginine-dependent	Arginine-, hypoxanthine- and uracil-dependent	Others
Localized	825	886	291 (33)	146 (16)	38 (4)	387 (44)	24 (3)
Disseminated	15	15	–	–	1 (7)	14 (93)	–
Total	840	901	291	146	39	401	24

genital or pharyngeal area in the remainder. Fourteen strains required arginine, hypoxanthine and uracil, and all but one also required leucine to grow. The requirement for arginine of all 14 strains was satisfied by ornithine. The difference between the two types of infection in the proportions of isolates requiring arginine, hypoxanthine and uracil was significant ($P < 0.0005$) by chi-square testing.

Discussion

Since Catlin's description of *Neisseria*-defined agar and confirmation of the stability of gonococcal auxotypes, the importance of auxotyping as an epidemiologic tool has been widely recognized.

As in all the other cities in which a similar study has been conducted, in Montreal more than 90% of all the gonococcal strains isolated belonged to four main auxotypes. The distribution of these auxotypes in the United States has been found to vary from one region to the next. A high percentage of the strains isolated in Montreal and Seattle required arginine, hypoxanthine and uracil, whereas in cities near Montreal, such as Boston, these strains were infrequent. The factors responsible for the regional differences are still unknown, but it seems that the strains requiring arginine, hypoxanthine and uracil are less often isolated from low-income patients, who frequently delay seeing a physician and take penicillin in subtherapeutic doses.[8] At hôpital Saint-Luc most gonococcal isolates are from middle-class students from a nearby university campus.

In Hamilton, Ont. Hendry and Stewart[13] conducted a similar study of gonococcal isolates but used a different system of classification, so that we could not strictly compare their findings with ours. Nevertheless, of their isolates prototrophs and strains requiring proline, or ornithine, uracil and hypoxanthine, or citrulline, uracil and hypoxanthine (the last two being equivalent to our strain requiring arginine, hypoxanthine and uracil) accounted for 14.0%, 10.6%, 25.6% and 7.4% respectively. The two sets of results therefore suggest that there are differences in auxotype distribution between Canadian cities.

Once the importance of the strain requiring arginine, hypoxanthine and uracil in localized gonococcal infections in our region was noted, we were interested in verifying the already reported association between this auxotype and disseminated gonococcal infection. This form of gonococcal infection is well known in Montreal, as a 1973 report by one of us of six cases stresses.[14] During the 34-month study period 15 patients with disseminated gonococcal infection were admitted to our hospital — 2% of all the patients with a gonococcal infection in our study. This frequency is in accord with the 1% to 3% reported in Seattle. Furthermore, 14 of the 15 infections were caused by strains requiring arginine, hypoxanthine and uracil, and only two patients were related to each other. In

a study from Seattle Knapp and Holmes[7] showed that 89% of the strains causing disseminated gonococcal infection required arginine, hypoxanthine and uracil. However, Eisenstein, Lee and Sparling[15] found that most strains causing such an infection were sensitive to penicillin while requiring arginine, hypoxanthine and uracil. We also found that strains with these requirements were very sensitive to both penicillin and tetracycline.[16] These findings reaffirm that disseminated gonococcal infection is due to gonococci with unique nutritional requirements. Most of the strains are reportedly resistant to the bactericidal action of serum and have low antibiotic resistance.[15,17,18] Although these strains are often responsible for disseminated gonococcal infection, this condition develops in only a small proportion of patients (3% in our study) infected with them. These phenotypic properties probably coincide in certain strains with other factors involved in virulence and invasiveness.

Gonococcal auxotyping helps to determine the history of an infection in patients and their contacts. It permits us to eliminate treatment failure as the cause of a second episode of gonorrhea when the auxotype responsible for the second episode is not the one responsible for the first, provided the patient was not initially infected with two auxotypes (as was the case for two of our patients), one of which proves resistant to the administered antibiotic. During our study 59 patients had two episodes of gonorrhea, and in 23 instances the interval between the episodes was 4 to 6 weeks: 7 of the 23 were infected with a different auxotype the second time; the other 16 had been reinfected by an untreated contact, had acquired a new infection with the same auxotype or had not been cured of the initial infection. If a patient is infected with a frequently encountered auxotype, such as that requiring arginine, hypoxanthine and uracil or a prototroph and returns a few weeks later with symptoms caused by the same auxotype, one cannot differentiate on the basis of auxotype alone between reinfection and treatment failure. On the other hand, if the auxotype is rare — for example, one that requires either methionine or arginine — chances are that this strain did not respond well to the prescribed antibiotic. Gonococcal susceptibility testing should help clarify the matter. Auxotyping can further help to evaluate the chances of successful treatment when an infection is caused by a given auxotype. Indeed, most strains requiring arginine, hypoxanthine and uracil are uniformly susceptible to most antibiotics used in the treatment of gonorrhea.

It would prove most useful if auxotyping results could be obtained from other investigators in Canada so that we could find out if differences in the regional distribution of auxotypes exist in our country and so that light can be shed on the responsible factors.

We thank Renée Mantha for technical assistance and Angela Gurd for typing the manuscript.

References

1. CATLIN BW: 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, 1973
2. CARIFO K, CATLIN BW: *Neisseria gonorrhoeae* auxotyping: differentiation of clinical isolates based on growth responses on chemically defined media. *Appl Microbiol* 26: 223, 1973
3. CATLIN BW, PACE PJ: Nutritional requirements and penicillin susceptibilities of gonococci from pharyngeal and anogenital sites. *Br J Vener Dis* 53: 299, 1977
4. CATLIN BW: Characteristics and auxotyping of *Neisseria gonorrhoeae*, in *Methods in Microbiology*, vol 10, BERGAN T, NORRIS JR (eds), Acad Pr, London, 1977, p 345
5. LASCOLEA LJ JR, YOUNG FE: Development of a defined minimal medium for the growth of *Neisseria gonorrhoeae*. *Appl Microbiol* 28: 70, 1974
6. CATLIN BW: Genetic transformation of biosynthetically defective *Neisseria gonorrhoeae* clinical isolates. *J Bacteriol* 120: 203, 1974
7. KNAPP JS, HOLMES KK: Disseminated gonococcal infections caused by *Neisseria gonorrhoeae* with unique nutritional requirements. *J Infect Dis* 132: 204, 1975
8. KNAPP JS, THORNSBERRY C, SCHOOLNIK GA, et al: Phenotypic and epidemiologic correlates of auxotype in *Neisseria gonorrhoeae*. *J Infect Dis* 138: 160, 1978
9. CRAWFORD G, KNAPP JS, HALE J, et al: Asymptomatic gonorrhea in men: caused by gonococci with unique nutritional requirements. *Science* 196: 1352, 1977
10. YOUNG H, PATERSON IC, MCDONALD DR: Rapid carbohydrate utilization test for the identification of *Neisseria gonorrhoeae*. *Br J Vener Dis* 52: 172, 1976
11. MORELLO JA, LERNER SA, BOHNHOFF M: Characteristics of atypical *Neisseria gonorrhoeae* from disseminated and localized infections. *Infect Immun* 13: 1510, 1976
12. STEERS E, FOLTZ EL, GRAVES BS, et al: An inocula replicating apparatus for routine testing of bacterial susceptibility to antibiotics. *Antibiot Chemother* 9: 307, 1959
13. HENDRY AT, STEWART IO: Auxanographic grouping and typing of *Neisseria gonorrhoeae*. *Can J Microbiol* 25: 512, 1979
14. TURGEON P, LAVERDIÈRE M, RICHER G, et al: Manifestations extra-génitales de la gonorrhée. *Union Med* 102: 1883, 1973
15. EISENSTEIN BI, LEE TJ, SPARLING PF: Penicillin sensitivity and serum resistance are independent attributes of strains of *Neisseria gonorrhoeae* causing disseminated gonococcal infection. *Infect Immun* 15: 834, 1977
16. TURGEON PL, JOLIVET GRANGER M, MANTHA R: Auxotypes and penicillin susceptibility of *Neisseria gonorrhoeae* isolated from localized and disseminated infections (abstr). *Can J Public Health* 71: 131, 1980
17. WIESNER PJ, HANDSFIELD HH, HOLMES KK: Low antibiotic resistance of gonococci causing disseminated infection. *N Engl J Med* 288: 1221, 1973
18. SCHOOLNIK GK, BUCHANAN TM, HOLMES KK: Gonococci causing disseminated gonococcal infection are resistant to bactericidal action of normal human sera. *J Clin Invest* 58: 1163, 1976