Reduction of Nitrite by *Neisseria gonorrhoeae*

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A total of 112 *Neisseria gonorrhoeae* strains representing protein I serogroups WI, WII, and WIII and six auxotypes were tested for their ability to reduce nitrite. All strains reduced 0.001% (wt/vol) potassium nitrite (KNO_2) within 24 h and 0.01% (wt/vol) KNO_2 within 48 h. None of the strains reduced 0.1% (wt/vol) KNO_2 even after incubation at 37°C for 5 days, and the strains could not be cultured from these tests after incubation for 24 h. Thus, all of the *N. gonorrhoeae* strains tested were nitrite sensitive but were able to reduce subtoxic concentrations of KNO_2 . Nitrite reduction is a species characteristic for *N. gonorrhoeae*.

Nitrite reduction is included as a differential test for the classification of *Neisseria* spp. and *Branhamella catarrhalis* (7, 9). Nitrite is reduced by all human *Neisseria* spp., with the possible exception of *Neisseria gonorrhoeae* strains and strains of some serogroups of *Neisseria meningitidis* (6, 7). In 1961, Berger showed that neither *N. gonorrhoeae* strains nor some *N. meningitidis* strains could reduce 0.1% (wt/vol) nitrite (1). While characterizing *Neisseria cinerea* strains in 1962, Berger and Paepcke demonstrated that although most *N. cinerea* strains reduced 0.1% (wt/vol) nitrite, 3 of 28 strains (11%) did not reduce 0.1% (wt/vol) nitrite but reduced 0.01% (wt/vol) nitrite (3). Berger subsequently reevaluated nitrite reduction by *N. gonorrhoeae* and *N. meningitidis* (2). All five strains of *N. gonorrhoeae* tested reduced 0.01% (wt/vol) nitrite. However, among 58 *N. meningitidis* strains tested, only strains of serogroups A, D, and Y could reduce 0.01% (wt/vol) nitrite, whereas strains of serogroups B, C, X, and Z could not. Unaware of the study of Berger (2), co-workers and I rediscovered that *N. gonorrhoeae* strains could reduce nitrite while we were evaluating nitrite reduction as a test for differentiating between clinical isolates of *N. cinerea* and *N. gonorrhoeae* (6). Since nitrite reduction by *N. meningitidis* strains was serogroup specific and three serogroups (serogroups WI, WII, and WIII) belonging to two protein I molecular types are recognized for *N. gonorrhoeae* (10, 11), I reevaluated nitrite reduction by *N. gonorrhoeae* strains to determine whether this is a species characteristic.

(The results were reported in part at the Annual Meeting of the American Society for Microbiology, New Orleans, La., March 1983 [J. S. Knapp, Abstr. Annu. Meet. Am. Soc. Microbiol. 1983, D27, p. 63].)

MATERIALS AND METHODS

Strains. A total of 112 *N. gonorrhoeae* strains were tested. The laboratory strains included the taxonomic type strain, strain NRL 30010 (= ATCC 19424), 15 serogroup WI, WII, and WIII serological reference strains (11), strains NRL 905 (AHU⁻/WI) and NRL 8038 (F62; Prö/WII), and strains isolated in The Philippines (4 strains), Europe (5 strains), and Africa (18 strains, 4 of which were β -lactamase positive). Also included were 67 cervical and urethral isolates from women and men attending an Adult Medicine Clinic in Seattle, Wash. The clinical isolates represented six auxotypes and serogroups WI, WII, and WIII (Table 1). Five *Neisseria mucosa* strains were compared with the *N. gonorrhoeae* strains in these tests. The strains were grown on supplemented GC base medium (Difco Laboratories, Detroit, Mich.), as described previously (13). Strains were stored at -70°C in a solution of 50% heat-inactivated (56°C,

30 min) gamma-globulin-free horse serum (GIBCO Laboratories, Grand Island, N.Y.) in tryptic soy broth (Difco).

Auxotyping and serogrouping of clinical isolates of *N. gonorrhoeae*. The isolates were auxotyped on Catlins NEDA defined medium, as described previously (4, 5). Serogrouping was performed by using group-specific polyclonal antisera in a coagglutination test, as described previously (11).

Nitrite reduction tests. All *N. gonorrhoeae* and *N. mucosa* strains were tested in 1-ml volumes of tryptic soy broth containing 0.1, 0.01, and 0.001% (wt/vol) KNO_2 in Pyrex tubes (16 by 100 mm; Corning Glass Works, Corning, N.Y.). The tests were inoculated in triplicate with loopfuls of an overnight culture grown on supplemented GC base agar at 37°C for 24 h in 5% CO_2 . A loopful of broth was removed from each test in 0.001% (wt/vol) KNO_2 , plated onto supplemented GC base medium, and incubated at 37°C for 24 h to confirm that positive tests resulted from *N. gonorrhoeae* activity. Similarly, each test in 0.1% (wt/vol) KNO_2 was tested to determine whether *N. gonorrhoeae* strains were viable after incubation at 37°C for 24 h. Tests for residual nitrite were made after incubation at 37°C without CO_2 for 24 h, 48 h, and 5 days, as described previously (12). Tests were recorded as positive only if nitrite was reduced completely.

RESULTS AND DISCUSSION

In accordance with the findings of Berger (1), all five *N. mucosa* strains reduced 0.1% (wt/vol) KNO_2 completely within 24 h. In contrast, none of the 112 *N. gonorrhoeae* strains reduced 0.1% (wt/vol) KNO_2 even after incubation for 5 days. All *N. gonorrhoeae* strains, irrespective of auxotype or serogroup, reduced 0.01% (wt/vol) KNO_2 within 48 h and 0.001% (wt/vol) KNO_2 within 24 h. Thus, all *N. gonorrhoeae* strains were nitrite sensitive, but reduced subinhibitory concentrations of nitrite.

Pure cultures of *N. gonorrhoeae* were isolated from all tests in medium which had contained 0.001% (wt/vol) KNO_2 . In contrast, *N. gonorrhoeae* was not isolated from tests in medium that contained 0.1% (wt/vol) KNO_2 and was tested after 24 h of incubation. Thus, the *N. gonorrhoeae* strains were not only unable to reduce, but were killed by, 0.1% (wt/vol) KNO_2 .

Detection of nitrite reduction by bacterial species is routinely made in a nutrient medium containing 0.1% (wt/vol) KNO_2 (12). Strains are tested in 4-ml volumes of medium in 15- by 125-mm tubes. Deep broth tubes are recommended because nitrite reduction occurs only in the absence of oxygen (8), and the ratio of surface area to volume is kept small to prevent oxygen from diffusing into the medium and inhibiting nitrite reduction. In this study, nitrite reduction

TABLE 1. Characteristics of 67 clinical isolates of *N. gonorrhoeae* tested for their ability to reduce nitrite

W serogroup ^a	No. with auxotype ^b					
	AHU ⁻	Proto	Pro ⁻	Arg ⁻	Pro ⁻ Arg ⁻	PAU ⁻
WI	5	2	1			
WII		39	10	1	1	2
WIII		4			1	1

^a Serogroups WII and WIII are subgroups which belong to the Protein IB molecule group, which is distinct from the protein 1A molecule group to which serogroup WI strains belong. (10, 11).

^b AHU⁻, Arginine, hypoxanthine, and uracil requiring; Proto, no requirements; Pro⁻, proline requiring; Arg⁻, arginine requiring; PAU⁻, proline, arginine, and uracil requiring. All strains required cystine-cysteine for growth.

was detected easily in 1-ml volumes of medium in 12- by 72-mm tubes. I postulated that *N. gonorrhoeae* cells rapidly reduced any dissolved oxygen in the medium and that cells near the medium surface continued to reduce any dissolving oxygen, thus permitting nitrite reduction to occur in the anoxygenic medium.

Although *N. gonorrhoeae* strains reduced subinhibitory nitrite concentrations, they were inhibited by, and could not reduce, the 0.1% (wt/vol) KNO₂ routinely recommended for nitrite reduction tests (12). The results obtained in this study emphasize the need to modify routinely recommended tests where necessary in order to determine whether nitrite-sensitive bacterial species can reduce nitrite at subinhibitory concentrations. Obviously, this applies not only to descriptions of new taxa, but also to reevaluation of well-established species, such as *N. gonorrhoeae*. Consequently, I recommend that nitrite reduction tests be made in a broth medium containing either 0.01 or 0.001% (wt/vol) KNO₂ in order to detect nitrite reduction by those bacterial species that are unable to reduce 0.1% (wt/vol) KNO₂.

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