Influence of Sodium Chloride on Growth of Neisseria meningitidis

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Nasopharyngeal isolates of Neisseria meningitidis were tested for growth on nutrient agar with and without the addition of 0.8% sodium chloride. Of the 822 strains tested, 1.3% grew on the salt-free medium, and 74.1% grew on the medium supplemented with sodium chloride.

In preparation for an extensive evaluation of the effect of rifampin on the nasopharyngeal carriage of Neisseria meningitidis in Marine Corps recruits (1), we adopted the basic primary isolation and identification schema of Devine et al. (6) as our laboratory routine. The schema utilizes the inability of N. meningitidis to grow on nutrient agar. Various sources support the validity of this technique (2, 4, 7). However, we found that many nasopharyngeal isolates of N. meningitidis grew on nutrient agar to a variable degree, in contradiction to the standard test procedure. Further investigation revealed that Devine (6) used nutrient agar (Difco no. 0001), whereas we used 1.5% nutrient agar (Difco no. 0069). The only difference was the inclusion of 0.8% NaCl in the latter formula. To determine whether the difference in the growth of N. meningitidis on the two types of nutrient agar was due to an osmotic effect or a requirement for either or both of the ions of sodium chloride, we tested the organisms for growth on nutrient agars containing sucrose, sodium chloride, potassium chloride, or sodium sulfate.

Routine meningococcal surveillance on Marine Corps trainees provided 822 strains of N. meningitidis for testing growth on nutrient agar (Difco no. 0001) with and without the addition of 0.8% sodium chloride. When growth was present on any of these media, colonies larger than 1 mm in diameter were produced after 24 hr of incubation at 37°C in an atmosphere of 8% carbon dioxide. The strains were isolated and identified by the method of Beam et al. (1). Carbohydrate fermentation reactions were determined by the Microtiter method of Davies et al. (5).

Of the 822 N. meningitidis strains tested, 11 strains (1.3%) grew without NaCl, while 609 strains (74.1%) grew with the addition of 0.8% NaCl. These results, tabulated by serogroup, are shown in Table 1.

To determine whether the growth of N. meningitidis on nutrient agar containing salt was due to an increased osmotic pressure of the medium, or a requirement for sodium chloride, 275 strains were tested on plain nutrient agar and on nutrient agar containing one of the following reagents: 0.14 M sodium chloride, 0.24 M sucrose, 0.14 M potassium chloride, or 0.10 M sodium sulfate. These concentrations are in the same ratio to isotonic solutions, as determined by Chase (3), as 0.8% sodium chloride is to 0.9% sodium chloride (isotonic). Of the 275 strains tested, 7 (2.5%) grew on plain nutrient agar, 229 (83.3%) grew with NaCl, 6 (2.2%) grew with sucrose, 160 (58.2%) grew with KCl, and 96 (34.9%) grew with Na₂SO₄.

These results indicate that growth of N. meningitidis on 1.5% nutrient agar (Difco no. 0069) is not due to increased osmotic protection provided by NaCl in the medium, but to a need in some strains for either the sodium or chloride ion and in some strains a need for both ions. When only one of the two ions was present, there was a significant reduction in the number of strains that grew compared to the number that grew in the presence of both ions.

A significantly smaller number of serogroup Y strains (P < 0.01) grew on nutrient agar containing NaCl than the other serogroups (Table 1). The reason for this is not clearly understood.

The inability of over 98% of meningococcal strains to grow on plain nutrient agar (containing only beef extract, peptone, and agar) is a valuable aid in the identification of these organisms. However, the substitution of 1.5%
**TABLE 1. Growth of *N. meningitidis* on nutrient agar with and without salt**

| No. of strains | Serogroup | Growth on nutrient agar** | Growth on nutrient agar + sodium chloride |
|----------------|-----------|---------------------------|-----------------------------------------|
|                |           | No. | %   | No. | %   |
| 134            | B         | 3   | 2.2 | 106 | 79.1|
| 280            | C         | 4   | 1.5 | 222 | 85.4|
| 297            | Y         | 3   | 1.0 | 170 | 57.2|
| 131            | Other     | 1   | 0.8 | 111 | 84.7|
| Total: 822     |           | 11  | 1.3 | 609 | 74.1|

* These strains also grew on nutrient agar plus sodium chloride.

** These serogroups include X, 135, 29E, RAS', RAS-10, and non-groupable.

“nutrient agar,” which contains 0.8% NaCl, for nutrient agar without NaCl will invalidate this test procedure.

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