Meningitis Caused by Maltose-Negative Variant of *Neisseria meningitidis*

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A maltose-negative variant of *Neisseria meningitidis*, Slaterus Y, was recovered from a patient with meningitis. A report of the case is presented and the medical-legal significance of such an isolate is briefly discussed.

Neisseria gonorrhoeae and *Neisseria meningitidis* are well-recognized pathogens in humans. Most laboratories identify the species of these organisms based upon their ability to utilize various carbohydrates in cystine-Trypticase agar (CTA). Both species typically produce acid from glucose and are sucrose and lactose negative. The sole biochemical reaction which distinguishes meningococci from gonococci is the former's ability to produce acid from maltose (4).

Occasional strains of *N. meningitidis* have been recovered from clinical specimens which give false-negative maltose reactions on CTA sugars. This phenomenon is well documented and has been most commonly attributed to nutritional deficiencies in the CTA basal medium which fail to support adequate growth of certain fastidious strains of meningococci to produce distinct biochemical reactions (29). In addition, some strains of meningococci have been reported to produce transient false-negative maltose reactions when first isolated, but they often acquire the ability to utilize maltose after several subcultures (11). Nonspecific fermentation reactions may also result from the prolonged incubation of CTA sugars (4). To minimize these problems a number of improved media formulations (2, 8, 14) and new techniques (16, 19, 20, 25, 31) have been developed for the identification of *Neisseria* based upon the utilization of various carbohydrates.

In 1967 Kingsbury (17) reported that 80% of meningococcal isolates obtained from transformation experiments and resistant to greater than 10 μg of sulfadiazine per ml were maltose negative when tested by conventional procedures. He also showed that these microorganisms were bona fide maltose-negative variants in that they lacked the necessary enzymes for maltose utilization. Since that study, we are not aware of any report in the English literature which documents the recovery of a genuine maltose-negative

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strain of *N. meningitidis* from a clinical specimen.

The purpose of this paper is to report the recovery of a bona fide maltose-negative variant of *N. meningitidis* from a patient with meningitis and to remind laboratorians of the medical-legal significance of such an isolate.

CASE REPORT

A 4-month-old male was admitted to Waterbury Hospital Health Center via the emergency room with a chief complaint of fever, irritability, and refusal to eat. The child was in apparent good health until 4 days before admission when he developed a dry, nonproductive cough and watery diarrhea. On the morning of admission the child awoke with a fever and chills, appeared irritable, and vomited several times.

Upon admission to the emergency room, the patient presented as a well-developed, well-nourished white male with a temperature of 104.2°F (ca. 40°C). Physical examination was within normal limits except that the neck seemed somewhat stiff, with the patient crying when flexion of the head was attempted. The right tympanic membrane was somewhat dull and injected when compared to the left, which seemed normal.

Laboratory examination showed the following values: hemoglobin 11.3 g/dl; hematocrit, 32.9%; and leukocyte count, 18,700 mm^3 (with 92% polymorphonuclear leukocytes [PMN] and 8% lymphocytes). Blood glucose was 84 mg/dl and electrolytes, blood urea nitrogen, and urinalysis were essentially within normal limits.

An admission lumbar puncture was performed; the fluid was clear and colorless with five leukocytes (80% PMNs, 20% lymphs), a glucose of 63 mg/dl, and a protein of 25 mg/dl. The spinal fluid was centrifuged, and examination of the sediment by Gram stain revealed no organisms. At 6 h after admission, a repeat spinal tap was performed; the fluid from this study was clear and colorless, with 14 leukocytes (40% PMNs and 60% lymphocytes), a glucose of 66 mg/dl, and a protein of 37 mg/dl. Gram stain of the spinal fluid sediment was negative for microorganisms. At this time the patient was placed on intravenous fluids and chloramphenicol.

A third spinal tap was performed 11 h after admission; this sample was cloudy and had a cell count of 4,715 leukocytes per mm^3 (92% PMNs, 8% lymphs), a

glucose of 25 mg/dl, and a protein of 276 mg/dl. Gram stain of the sedimented spinal fluid showed intracellular gram-negative diplococci. The chloramphenicol was discontinued and the patient was started on intravenous penicillin G (400,000 U every 3 h).

An oxidase-positive, gram-negative diplococcus was recovered from the second and third spinal fluid cultures; whereas there was no growth from the admission lumbar tap. In addition, a similar type of organism was recovered from cultures of the patient's nasopharynx, throat, and blood (×2) collected at the time of admission. Each isolate was subsequently identified as a maltose-negative variant of *N. meningitidis*, Slaterus Y (24), which was resistant to sulfadiazine by the disk diffusion method (10).

The patient was continued on intravenous penicillin G for 8 days, made an uneventful recovery, and was discharged 10 days after admission.

RESULTS AND DISCUSSION

During the patient's period of hospitalization, epidemiological surveillance cultures were collected from close family contacts, including the mother, father, and maternal grandparents. A maltose-negative *N. meningitidis*, Slaterus Y, was recovered from the nasopharynx of only the father, and it is possible that he served as the carrier for the patient's infecting organism.

Each of the meningococcal isolates recovered from the patient's spinal fluid, blood, nasopharynx, and throat, as well as the father's nasopharynx, produced luxuriant colonial growth (1.5 to 2.0 mm in diameter) on chocolate agar plates after 24 h of incubation at 35°C with 5% carbon dioxide atmosphere. The colonies were nonpigmented, and the morphology was round and smooth. Carbohydrate utilization was tested on conventional CTA and Columbia agar base with 1% phenol red. Each isolate produced acid from glucose but not maltose, sucrose, or lactose after 96 h of incubation. A similar biochemical pattern was observed when each of the isolates was tested by the Bactec radiometric procedure (25).

Although the biochemical results from each of these methods was consistent for *N. gonorrhoeae*, this identification was questioned since the colonial morphology and luxuriant growth were more typical for *N. meningitidis*. Attempts to serogroup the isolates with meningococcal antisera were unsuccessful since each of the organisms autoagglutinated in saline.

The isolates were sent to the Connecticut State Department of Health and the Center for Disease Control for confirmation. The strains were identified as *N. meningitidis* Slaterus Y by an immunofluorescent technique, and the unusual maltose-negative characteristic of these variants was confirmed. For determining the sugar utilization patterns of these isolates, the Con-

necticut State Department of Health employed an in-house basal medium preparation consisting of a proteose-peptone no. 3-beef extract agar base supplemented with cornstarch, a 1% final concentration of the test carbohydrate, and phenol red as the indicator, whereas the Center for Disease Control used the rapid fermentation method described by Kellogg and Turner (16). In addition, all isolates were found to be resistant to sulfadiazine by the disk diffusion method (10).

Attempts were not made to determine the biochemical defect responsible for the inability of these strains to utilize maltose. However, Kingsbury (17) showed by genetic studies that the relationship between maltose negativity and sulfadiazine resistance was associated with only one mutation or the transfer of one gene. Furthermore, enzymatic studies of each of his maltose-negative, sulfadiazine-resistant strains showed that these isolates lacked either maltose permease or maltose phosphorylase activity. Since the isolates reported in the present study were sulfadiazine resistant, it is postulated that they lacked either of the enzymes necessary for maltose utilization.

Traditionally, *N. gonorrhoeae* and *N. meningitidis* were thought to occupy separate and distinct anatomical sites and cause different infectious processes. Within recent years it has become increasingly apparent that these microorganisms are not restricted by any particular anatomical boundary and may produce similar infections (12). Gonococci may be recovered from extragenital sites where they are well-known causes of meningitis (15, 26, 27), septic arthritis (6, 15), and pharyngitis (3, 13), as well as carried in the oropharynx without producing overt disease (3, 23). Furthermore, meningococci may be recovered from genitourinary specimens where they may cause infection (1, 7, 21, 30) or live as a commensal (5, 9, 18).

Since it is no longer possible to associate the recovery of a particular *Neisseria* species to a specific disease process of an anatomical site, it has become increasingly important for clinical microbiology laboratories to utilize appropriate techniques to accurately characterize species of *Neisseria*. Although considerable progress has been made to minimize the problems of the false-negative maltose reactions of meningococci, the emergence of bona fide maltose-negative variants poses a serious problem for laboratories that depend solely upon carbohydrate utilization for the identification of *Neisseria*. Such a practice could result in an erroneous identification of a maltose-negative meningococcus as *N. gonorrhoeae*. Clearly, such an error presents a major problem of social and epide-

miological importance to patients and health authorities. In addition, the medical-legal consequences of such an error for the clinician and the diagnostic laboratory are obvious.

Although the actual incidence of maltose-negative strains of *N. meningitidis* is unknown, laboratorians are cautioned about the existence of this biochemical variant. Several months after the hospital discharge of the 4-month-old patient, another maltose-negative variant of meningococcus was recovered from a nasopharyngeal carrier seen in our outpatient clinic. The isolate was identified by immunofluorescence as *N. meningitidis* group C, which was also resistant to sulfadiazine.

Clinical microbiologists may become suspicious that they are working with a maltose-negative variant of *N. meningitidis* based upon the colonial morphology of the isolate. The typical colonial morphologies of *N. gonorrhoeae* and *N. meningitidis* are markedly different (11). Gonococci are usually slow growers and produce smaller colonies which are often opaque, greyish-white, glistening, and convex; meningococci, on the other hand, normally produce larger colonis that are round, smooth, and practically nonpigmented. In our experience, these criteria were useful in arousing our suspicions that we might be dealing with a biochemical variant of *N. meningitidis*.

Additional studies need to be performed to determine the incidence of bona fide maltose-negative variants of *N. meningitidis* to identify the magnitude of this problem. Although immunofluorescence was used to confirm the identity of these strains, this technique may not offer an immediate and practical solution to this problem since the technology is not without limitations (22, 28) and is not in widespread use in most diagnostic laboratories. Since most laboratories identify *Neisseria* based upon carbohydrate utilization, it is conceivable that alternative methodology may need to be developed to reliably distinguish meningococci from gonococci.

LITERATURE CITED

1. **Beck, A., J. L. Fluker, and D. J. Platt.** 1974. *Neisseria meningitidis* in urogenital infection. Br. J. Vener. Dis. **50**:367–369.
2. **Beno, D. W., L. F. Devine, and G. L. Larson.** 1968. Identification of *Neisseria meningitidis* carbohydrate fermentation patterns in Mueller-Hinton broth. J. Bacteriol. **96**:563.
3. **Bro-Jorgensen, A., and T. Jensen.** 1971. Gonococcal tonsillar infections. Br. Med. J. **4**:660–661.
4. **Catlin, B. W.** 1974. *Neisseria meningitidis* (meningococcus), p. 116–123. *In* E. H. Lennette, E. H. Spaulding, and J. P. Truant (ed.), Manual of clinical microbiology, 2nd ed. American Society for Microbiology, Washington, D. C.
5. **Chapel, T. A., C. Gatewood, and M. B. Keane.** 1977. *Neisseria meningitidis* in the anal canal of homosexual men. J. Infect. Dis. **136**:810–812.
6. **Cooke, C. L., D. S. Owen, Jr., R. Irby, and E. Toone.** 1971. Gonococcal arthritis. J. Am. Med. Assoc. **217**:204–205.
7. **Fallon, R. J., and E. T. Robinson.** 1974. Meningococcal vulvovaginitis. Scand. J. Infect. Dis. **6**:295–296.
8. **Faur, Y. C., M. H. Weisburd, and M. E. Wilson.** 1975. Carbohydrate fermentation plate medium for confirmation of *Neisseria* species. J. Clin. Microbiol. **1**:294–297.
9. **Faur, Y. C., M. H. Weisburd, and M. E. Wilson.** 1975. Isolation of *Neisseria meningitidis* from the genitourinary tract and anal canal. J. Clin. Microbiol. **2**:178–182.
10. **Federal Register.** 1972. Rules and regulations. Antibiotic susceptibility discs. Fed. Regist. **37**:20525–20529.
11. **Feldman, H. A.** 1970. *Neisseria* infections other than gonococcal, p. 135–152. *In* H. L. Bodily, E. L. Updyke, and J. O. Mason (ed.), Diagnostic procedures for bacterial mycotic and parasitic infections, 5th ed. American Public Health Association, Inc., New York.
12. **Feldman, H. A.** 1971. Meningococcus and gonococcus: never the twain . . . well, hardly ever. N. Engl. J. Med. **285**:518–520.
13. **Fiumara, N. J., M. M. Wise, Jr., and M. Many.** 1967. Gonorrheal pharyngitis. N. Engl. J. Med. **276**:1248–1250.
14. **Flynn, J., and S. A. Waitkins.** 1972. A serum-free medium for testing fermentation reactions in *Neisseria gonorrhoeae*. J. Clin. Pathol. **25**:525–527.
15. **Holmes, K. K., G. W. Counts, and H. N. Beaty.** 1971. Disseminated gonococcal infection. Ann. Intern. Med. **74**:979–993.
16. **Kellogg, D. S., Jr., and E. M. Turner.** 1973. Rapid fermentation confirmation of *Neisseria gonorrhoeae*. Appl. Microbiol. **25**:550–552.
17. **Kingsbury, D. T.** 1967. Relationship between sulfadiazine resistance and the failure to ferment maltose in *Neisseria meningitidis*. J. Bacteriol. **94**:557–561.
18. **Lewis, J. F., and J. J. Alexander.** 1974. Isolation of *Neisseria meningitidis* from the vagina and cervix. Am. J. Clin. Pathol. **61**:216–217.
19. **Morse, S. A., and L. Bartenstein.** 1976. Adoption of the minitek system for the rapid identification of *Neisseria gonorrhoeae*. J. Clin. Microbiol. **3**:8–13.
20. **Reddick, A.** 1975. A simple carbohydrate fermentation test for identification of the pathogenic *Neisseria*. J. Clin. Microbiol. **2**:72–73.
21. **Reiss-Levy, E., and J. Stephenson.** 1976. Vaginal isolation of *Neisseria meningitidis* in association with meningococcaemia. Aust. N. Z. J. Med. **6**:487–489.
22. **Reyn, A.** 1965. Laboratory diagnosis of gonococcal infections. Bull. W.H.O. **32**:449–469.
23. **Shahidullah, M.** 1976. Pharyngeal gonorrhoea in homosexual males. Br. J. Vener. Dis. **52**:168–169.
24. **Slaterus, K. W.** 1961. Serological typing of meningococci by means of microprecipitation. Antonie Van Leeuwenhoek J. Microbiol. Serol. **27**:305–315.
25. **Strauss, R. R., J. Holderbach, and H. Friedman.** 1978. Comparison of a radiometric procedure with conventional methods for identification of *Neisseria*. J. Clin. Microbiol. **7**:419–422.
26. **Swierczewski, J. A., E. J. Mason, P. B. Cabrera, and M. Liber.** 1970. Fulminating meningitis with Waterhouse-Fridericksen syndrome due to *Neisseria gonorrhoeae*. Am. J. Clin. Pathol. **54**:202–204.
27. **Taubin, H. L., and L. Landsberg.** 1971. Gonococcal

meningitis. N. Engl. J. Med. **285**:504–505.

28. **Tramont, E. C., J. C. Sadoff, and M. S. Artenstein.** 1974. Cross reactivity of *Neisseria gonorrhoeae* and *Neisseria meningitidis* and the nature of antigens involved in the bactericidal reaction. J. Infect. Dis. **130**: 240–247.

29. **White, L. A., and D. S. Kellogg, Jr.** 1965. An improved fermentation medium for *Neisseria gonorrhoeae* and other *Neisseria*. Health Lab. Sci. **2**:238–241.

30. **Willmott, F. E.** 1976. Meningococcal salpingitis. Br. J. Vener. Dis. **52**:182–183.

31. **Young, H., I. C. Paterson, and D. R. McDonald.** 1976. Rapid carbohydrate utilization test for identification of *Neisseria gonorrhoeae*. Br. J. Vener. Dis. **52**:172–175.