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# Extracellular Streptococcal Neuraminidase

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Neuraminidases (sialidases) found in mammalian tissues and associated with viruses and bacteria selectively cleave terminal sialic acid residues from glycoproteins, glycolipids, and oligosaccharides (M. E. Rafelson et al., p. 171, *in* E. A. Balazs and R. W. Jeanloz [ed.], *The amino sugars*, vol. 2B, Academic Press, Inc., New York, 1966). These enzymes have been reported in the following bacteria: *Vibrio cholerae* (F. M. Burnet, et al., Brit. J. Exptl. Pathol. **27**:228, 1946); *Clostridium perfringens* (J. F. McCrea, Australian J. Exptl. Biol. Med. Sci. **25**:127, 1947); *C. tertium* (C. Howe et al., J. Bacteriol. **74**:365, 1957); *Pseudomonas fluorescens*, *P. stutzeri*, and *Lactobacillus bifidus* (M. Shilo, Biochem. J. **66**:48, 1957); and *Diplococcus pneumoniae* (C. M. Chu, Nature **161**:606, 1948; R. Heimer and K. Meyer, Proc. Natl. Acad. Sci. U.S. **42**:728, 1956). At the time this note was in preparation, S. Hayano and A. Tanaka (J. Bacteriol. **93**:1753, 1967) reported a group K streptococcal strain which produced extracellular neuraminidase, and strains of groups F, D, and M which broke down added bovine submaxillary mucin during growth.

There appears to be general agreement that neuraminidase in human saliva originates, at least in part, in the bacterial oral flora (S. A. Leach, Nature **199**:486, 1963; Y. Mishiro and K. Kirimura, J. Dental Res. **43**:1258, 1964; G. Rolla, Acta Odontol. Scand. **24**:431, 1966; M. J. Perlitsch and I. Glickman, J. Dental Res. **45**:1239, 1966), and neuraminidase activity has been demonstrated in mixed cultures of organisms indigenous to the human oral cavity (J. D. Thonard et al., J. Bacteriol. **89**:924, 1965). The present work shows that some pure strains of oral streptococci, as well as a group E strain, synthesize extracellular neuraminidase during growth.

Bacterial cultures maintained on slants of Todd-Hewitt broth (Fisher Scientific Co., Pittsburgh, Pa.) containing 1% agar and 5% whole sheep blood were transferred every 3 weeks. Liquid cultures were grown anaerobically in 10 ml of 3% Todd-Hewitt broth in the presence of $CO_2$ for 16 hr at 37 C.

Neuraminidase activity was assayed in liquid culture supernatant fluids by release of sialic acid, as measured by the method of L. Warren (p. 463, *in* S. P. Colowick and N. O. Kaplan [ed.], *Methods in enzymology*, vol. 6, Academic Press, Inc., New York, 1963), with *N*-acetylneuraminyllactose (100 $\mu$g/ml) as substrate. This substrate preparation has been described (M. Schneir and M. E. Rafelson, Biochim. Biophys. Acta **130**:1, 1967) as containing about 71% of 2,3-*N*-acetyl-neuraminyllactose and 14% of the 2,6 isomer.

Our investigations established that extracellular neuraminidase activity was present consistently when the streptococcal cultures were grown in Todd-Hewitt broth without any additions. Adding glucose (2%) during growth inhibited the expression of enzyme activity completely, and the addition of solid $CaCO_3$ to stabilize culture *p*H did not prevent the inhibition. Enzyme activity in anaerobic cultures was from one to five times higher than in aerobic cultures.

Preliminary study of the characteristics of the neuraminidase activity in culture filtrates showed that: (i) storage at 5 C for 36 days did not diminish activity; (ii) dialysis against dilute phosphate buffer, *p*H 6.5, increased activity by as much as two times; (iii) addition of $Ca^{++}$ (1 mM) did not increase activity; (iv) ethylenediaminetetraacetate addition, up to 2 mM, decreased activity markedly; (v) the activity was *p*H-dependent, with maximal activity in the range *p*H 6.3 to 7.0; (vi) heating at 56 C for 15 min destroyed 90% of the activity; (vii) addition of large amounts of *N*-acetylneuraminic acid (up to 0.40 $\mu$mole) did not inhibit activity; (viii) no activity was found in cell-free extracts of cells from 16-hr cultures, but their supernatant fluids were active; and (ix) enzymes degrading *N*-acetylneuraminic acid, such as *N*-acetylneuraminic acid aldolase, were absent in 11 strains which were tested.

A number of streptococcal strains were sur-

veyed for production of extracellular neuraminidase. Table 1 shows the strains, their sources, and the results of the survey. Only 8 of the 39 strains were positive for the enzyme and only 2 of the 8 are cariogenic in gnotobiotic animals (strains 167 and SL). All the positive strains were oral isolates from humans, except the group E strain. No correlation is apparent between extracellular neuraminidase production and cariogenicity in the positive strains.

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TABLE 1. *Extracellular neuraminidase in streptococcal cultures*

| Streptococcal strains tested[a] | Activity (μmoles per ml per 4 hr) | Cariogenic strains |
|---|---|---|
| 1. Group A, strain S23 | 0 | |
| 2. Group B, ATCC 12403 | 0 | |
| 3. Group C, strain 26RP66 | 0 | |
| 4. Group E, ATCC 12390 | 1.5 | |
| 5. Group F, ATCC 12392 | 0 | |
| 6. Group G, ATCC 12395 | 0 | |
| 7. Group H, ATCC 12399 | 0 | |
| 8. Group K, ATCC 12399 | 0 | |
| 9. Group O, ATCC 11843 | 0 | |
| 10. *Streptococcus sanguis* ATCC 10556 | 0 | |
| 11. *S. sanguis* ATCC 10557 | 0 | |
| 12. *Lactobacillus casei* ATCC 4646 | | S. Rosen and W. S. Lenny, Meeting Intern. Assoc. Dental Res., 44th 1966 |
| 13. *S. mitis* ATCC 903 | 0 | |
| 14. *S. sanguis* Hockley | 0 | |
| 15. *S. sanguis* Challis | 0 | |
| 16. *S. sanguis* | 0 | |
| 17. *S. sanguis* | 0 | |
| 18. *Streptococcus* strain 20 | 1.4 | |
| 19. *Streptococcus* strain 134 | 1.4 | |
| 20. *Streptococcus* strain 149 | 0.4 | |
| 21. *Streptococcus* strain 151 | 0 | |
| 22. *Streptococcus* strain 162 | 0 | |
| 23. *Streptococcus* strain 164 | 0 | |
| 24. *Streptococcus* strain 167 | 1.6 | S. Rosen, *personal communication* |
| 25. *Streptococcus* strain 169 | 1.4 | |
| 26. *Streptococcus* strain 171 | 0.4 | |
| 27. *Streptococcus* strain 174 | 0 | |
| 28. *Streptococcus* strain Enole | 0 | |
| 29. *Streptococcus* strain F90A | 0 | |
| 30. *Streptococcus* strain SBE | 0 | |
| 31. *Streptococcus* strain HS-6 | 0 | R. J. Fitzgerald and P. H. Keyes, J. Am. Dental Assoc. **61**:9, 1960 |
| 32. *Streptococcus* strain FA-1 | 0 | R. J. Fitzgerald et al., J. Dental Res. **39**:923, 1960 |
| 33. *Streptococcus* strain SL | 1.2 | P. H. Keyes, *personal communication* |
| 34. *Streptococcus* strain 25QR4R | 0 | R. J. Fitzgerald, *personal communication* |
| 35. *Streptococcus* strain PK-1 | 0 | R. J. Gibbons et al., Arch. Oral Biol. **11**:549, 1966 |
| 36. *Streptococcus* strain GS-5 | 0 | R. J. Gibbons et al., Arch. Oral Biol. Biol. **11**:549, 1966 |
| 37. *S. faecalis*, ND 405 | 0 | M. Wagner, Bacteriol. Proc., p. 99, 1967 |
| 38. *S. salivarius* | 0 | |
| 39. *Pseudomonas aeruginosa* | 0 | |

[a] Sources of strains tested: (1) R. C. Lancefield, Hospital of the Rockefeller Foundation, New York, N. Y.; (3) R. M. Krause, Hospital of the Rockefeller Foundation, New York, N. Y.; (2, 4–13) American Type Culture Collection, Washington, D.C.; (14–30) I. L. Shklair, Naval Dental Research Institute, Great Lakes, Ill.; (31–33) P. H. Keyes, National Institute for Dental Research, Bethesda, Md.; (34–36) R. J. Gibbons, Forsyth Dental Center, Boston, Mass.; (37) M. Wagner, Notre Dame University, South Bend, Ind.; (38 and 39) oral isolates from this laboratory. Strains 16–39 are oral isolates.