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# Neuraminidase Activities of Clinical Isolates of *Diplococcus pneumoniae*

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In a recent study, we found neuraminidase activity to be present in only 7 of 15 cultures of various types and strains of laboratory-adapted *Diplococcus pneumoniae* (R. T. Kelly, D. Greiff, and S. Farmer, J. Bacteriol. **91**:601, 1966). To determine whether a correlation existed between the presence of neuraminidase activity in pneumococci and human pneumococcal illness, we have studied freshly isolated *D. pneumoniae* from a variety of illnesses.

Clinically significant pneumococcal isolates were received from five different hospitals over a 6-month period. The organisms, identified by the optochin disc technique, were isolated from throat, sputum, blood, cerebrospinal fluid, external auditory canal, and conjunctival cultures. Each isolate was inoculated into 100 ml of Todd-Hewitt broth and incubated at 37 C for 18 hr; the organisms were collected after centrifugation at 5,000 $\times$ *g* for 15 min. Supernatant fluids were discarded, and the pneumococci were resuspended in 2 ml of distilled water, frozen (−65 C) and thawed (+20 C), and centrifuged at 20,000 $\times$ *g* for 15 min. Neuraminidase activities of the supernatant fluids were determined by incubation with *N*-acetylneuraminlactose for 30 min at 37 C, and the amount of *N*-acetylneuraminic acid liberated was measured (L. Warren, J. Biol. Chem. **234**:1971, 1959). The reacting mixture (0.25 ml) contained 0.1 ml of supernatant fluid, tris(hydroxymethyl)aminomethane-acetate buffer (*p*H 6.5) at a final concentration of 0.1 M, and 0.25 $\mu$M *N*-acetylneuraminlactose. *N*-acetylneuraminlactose was prepared from bovine colostrum by the method of M. Schneir, R. J. Winzler, and M. K. Rafelson, Jr. (Biochem. Prep. **9**:1, 1962). Suitable solutions of enzyme and substrate served as blank controls.

In contrast to our previous studies with laboratory strains, all of the 77 clinical isolates of this investigation (sputum, 64; blood, 5; cerebrospinal fluid, 4; throat, 2; conjunctiva, 1; external auditory canal, 1) possessed neuraminidase activity.

Although the capsular type of each isolate was not determined, studies by R. Austrian (Am. J. Med. Sci. **238**:133, 1959) would indicate that the 77 isolates represented a heterogenous population of capsular types. The presence of neuraminidase activity in all freshly isolated pneumococci suggests that the enzyme is essential for the metabolism of pneumococci within the natural host. The absence of the enzyme in many of the laboratory strains of pneumococci may reflect mutation during continuous cultivation of the organisms on artificial media which resulted in a selection of variants lacking enzyme activity.

Neuraminidase has been found in a variety of pathogenic bacteria and in the myxoviruses (M. E. Rafelson, Jr., Exposes Ann. Biochem. Med. **24**:121, 1963). In experimental influenzal encephalitis, viral neuraminidase, by splitting sialic acid from gangliosides and other sialic acid-containing compounds of the brain, was proposed as the mechanism responsible for the deaths of animals (R. T. Kelly and D. Greiff, Biochim. Biophys. Acta **115**:244, 1965). Histochemical studies of fatal pneumococcal infections in man and in experimental animals have demonstrated that the sialic acid content of infected tissues was reduced significantly (R. T. Kelly and D. Greiff, *in preparation*). These data suggested that the changes observed resulted from the cleavage by pneumococcal neuraminidase of terminal sialic acids from glycolipids and glycoproteins, components essential to many tissues and body fluids.

Recent data have shown that the enzymatic cleavage of sialic acid from the sialic acid-glycoprotein conjugates of cell membranes alters the charge at the surface of the plasma membrane and modifies or inhibits active cation transport (P. Emmelot and C. J. Bos, Biochim. Biophys. Acta **115**:244, 1966; J. L. Glick and S. Githens III, Nature **208**:88, 1965). The removal of sialic acid from membranes through the action of pneumococcal neuraminidase may, therefore, cause injury to the cells of infected tissue by the mechanisms above. The results of the present study that all freshly isolated pneumococci from human infection possess neuraminidase activity

suggest that neuraminidase is a significant factor in the biochemical pathology of pneumococcal infection.

The data of the studies above and the results of our previous investigations showing that encapsulated strains of pneumococci lacking neuraminidase activity were virulent for mice when inoculated intraperitoneally (R. T. Kelly, D. Greiff, and S. Farmer, J. Bacteriol. **91**:601, 1966), indicate the importance of the manner of infection for the determination of the virulence of microorganisms.

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