Brief Definitive Report

THE CAPSULE OF THE GONOCOCCUS*

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The presence or absence of capsules on *Neisseria gonorrhoeae* has been argued for many years. In 1921, Israeli (1) summarized opinions on encapsulation of gonococci as follows: "Some hold that it does possess a capsule, some question its existence, and some flatly deny that this organism has a capsule." In the intervening years, the existence of a gonococcal capsule has had both proponents (1–7) and opponents (8–11) but no definite resolution of the question. The present study was undertaken to try to clarify the controversy by utilizing numerous staining techniques for light microscopic evaluation of the presence or absence of capsules in several recently isolated as well as long-term serially passaged strains of gonococci. Our findings confirm the presence of capsules on *Neisseria gonorrhoeae*.

Materials and Methods

Clinical isolates of *N. gonorrhoeae* were obtained as cultures on Thayer-Martin agar from the Salt Lake City County Health Department. The organisms were subsequently passaged on typing agar (GC agar base formulation of Baltimore Biological Laboratory (BBL) modified to contain half the recommended amount of Trypticase Peptone) plus 1% IsoVitaleX (BBL) and were identified as gonococci by Gram stain, oxidase reaction, and sugar oxidation. These isolates, along with the strains MS11 and F62 which have been serially passaged in this laboratory for over 4 yr, were incubated at 35°C in 5% CO_2. Cultures were also frozen at −70°C in Trypticase Soy Broth (BBL) containing 40% (vol/vol) glycerol to maintain stocks.

Capsule stains that were used as originally described include the following: India ink wet mount (12); India ink smear and stain (13, 14); Hiss copper sulfate (15); Hiss potassium carbonate (15); sodium caseinate-methyl violet (16); Wright's stain (17); eosin-serum (18); flagella-capsule stain (19); Congo red-methylene blue (20); Alcian blue negative stain (21); Moller's negative stain (22); and Gram capsule stain (23). The Alcian blue positive capsule stain (24) was modified by conversion of bound Alcian blue to Monastral fast blue (25, 26). Both Pelikan (Gunther Wagner) and Higgins (Faber-Castell) India inks were used in India ink wet mounts and both were satisfactory.

Several types of shearing forces were used to remove capsules. These include ejecting suspensions of gonococci through a 25-gauge needle, shaking suspensions of organisms with glass beads, and sonication. The affect of these treatments was assessed in India ink wet mounts (12) or by Congo red negative staining (20).

Results

In all 16 strains of gonococci isolated from clinical specimens for this study and in the two multiply passaged laboratory strains, capsules are demonstrable. Of

* This work was supported by Public Health Service research grant AI-11073 to Dr. John Swanson from the National Institute of Allergy and Infectious Diseases.

the 14 capsule demonstration methods used for attempted visualization of capsules on *N. gonorrhoeae, Neisseria meningitidis*, and *Streptococcus pneumoniae*, India ink wet mounts provided the most consistent demonstration of capsules and were technically the simplest. Fig. 1 A–F depict comparative capsule demonstrations for *N. gonorrhoeae* (Fig. 1 A–C), *N. meningitidis* (Fig. 1 D–E), and *S. pneumoniae* (Fig. 1 F) in India ink wet mounts. Though the gonococcal capsule showed some variability in size among different preparations, it was consistently as easily discerned as that of meningococci but not so large as that of pneumococci. Usually the gonococcal capsule was approximately one to two times the diameter of the organism's cell body.

Positive capsule stains were less effective in demonstrating gonococcal capsules as there was an apparent shrinkage of the capsule due to dehydration by the staining fluids. These methods rely on color differences between the body of the bacterium and the capsule which is difficult to demonstrate with photomicrographs (Fig. 1 G–J).

Several enzymatic treatments (trypsin, chymotrypsin, lysozyme, hyaluronidase, neuraminidase, and glucuronidase) failed to modify the appearance of the gonococcal capsule. Heat (5 min in boiling water bath) also failed to remove capsules from these organisms. Shearing was quite effective in disrupting gonococcal capsulation as shown by comparison of Fig. 1 K and L. In these preparations stained by the Congo red method (26), both encapsulated and nonencapsulated organisms are seen after application of shearing. Apparent "free" capsules devoid of bacterial cell bodies can also be seen after shearing and constitute a strikingly different appearance as compared to nonsheared control preparations.

Capsules seemed to be most prominent in organisms derived from gonococcal colonies that had a mucoid appearance and behavior. Encapsulation also appeared to be seen best on organisms that are recently derived from a patient source. Some diminution of capsule size was apparent on repeated passage of these organisms, but capsules were clearly demonstrable not only after serial passage of these recent isolates but also on organisms that had been serially passaged for many years in our laboratory. Gonococci derived from colonies corresponding to all four of Kellogg's colony types (9) and aggregation variants (27) had demonstrable capsules as did gonococci that exhibit differing "leukocyte-association" reactivities (28). This result is in contrast to the report of Yamada and Sadoff (7) which indicates LA^+ organisms lack capsules, and as a result have increased attachment-ingestion by polymorphonuclear leukocytes.

Although the percentage of encapsulated organisms in a given strain or substrain preparation varied, and although the apparent size and quality of capsules varied in differing preparations, it should be emphasized that no strains studied were totally devoid of capsules.

Gonococcal capsules were positively stained by Alcian blue (Fig. 1 J), but this is not readily appreciated in black and white photomicrographs. Such Alcian blue stainability suggests that the capsular material is polysaccharide in nature.

Discussion

Our studies clearly show that *N. gonorrhoeae* is encapsulated. It is our

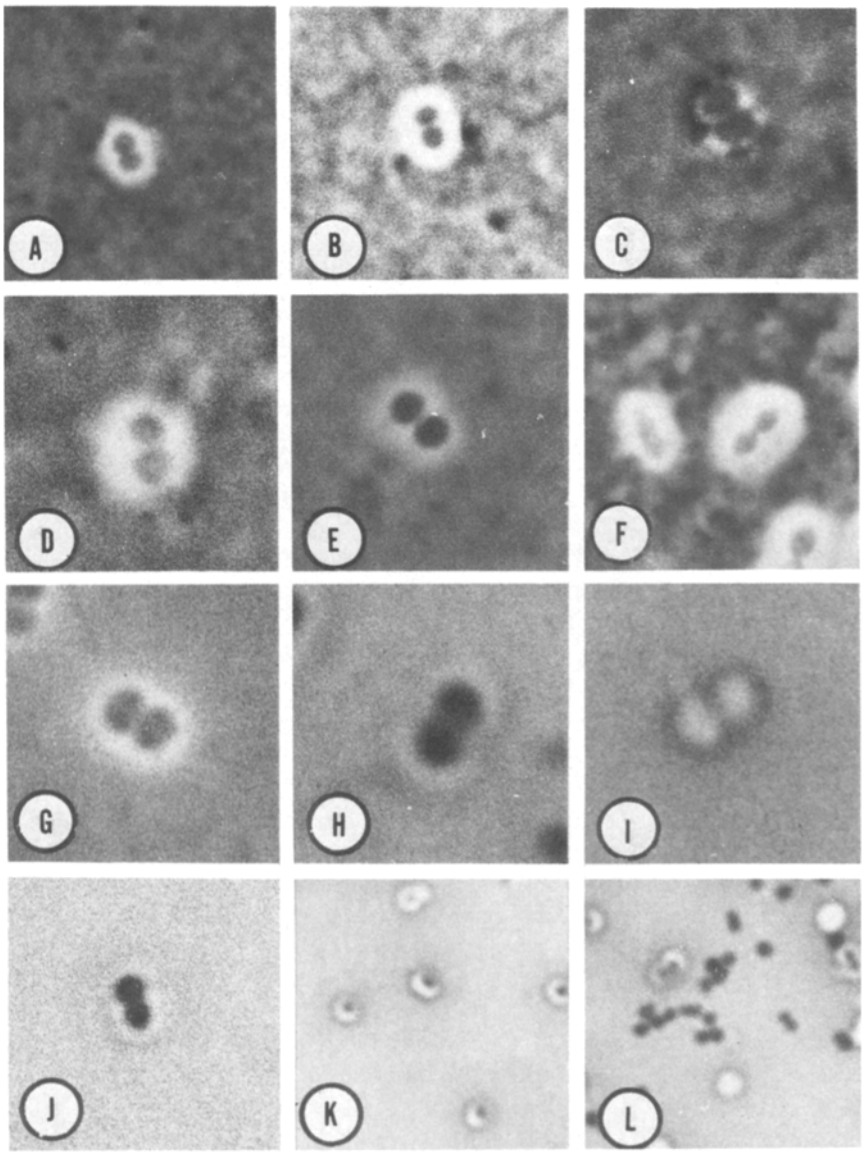

FIG. 1. Capsule demonstration. (A) India ink wet mount GC 762 (× 3,000). (B) India ink wet mount GC 762 (× 3,000). (C) India ink wet mount GC 762 (× 6,000). (D) India ink wet mount *N. meningitidis* group A (× 6,000). (E) India ink wet mount *N. meningitidis* group A (× 6,000). (F) India ink wet mount *S. pneumoniae* type III (× 2,500). (G) Churchman and Emellianoff (× 6,000): bacteria blue; capsule redish pink. (H) Hiss CO_3 (× 7,500): bacteria dark red; capsules white-pink. (I) eosin-serum (× 6,000): bacteria unstained; capsules red. (J) modified Novelli (× 4,500): bacteria red; capsule green. (K) Whites stain preshearing (× 2,000); bacteria blue; capsules clear. (L) Whites stain postshearing (× 2,000): bacteria blue; capsules clear.

opinion that previous discordant results regarding encapsulation of this organism is largely based on the seemingly fragile nature of the gonococcal capsule and its susceptibility to removal by mechanical forces. Alcian blue stainability of the gonococcal capsule suggests it is polysaccharide, but the chemical compo-

sition, the immunological character, and the possible biological role of this structure await clarification.

Summary

16 strains of *Neisseria gonorrhoeae* were subjected to several established techniques for capsule demonstration by light microscopy. In all strains examined, encapsulation of the gonococcus was demonstrated. Although the capsules were somewhat more easily seen in strains recently isolated from clinical material, organisms that had been passaged for several years also were encapsulated as were all the colony types within these strains. The gonococcal capsule is easily disrupted by mechanical forces.

We appreciate the help of Midge Beckman in preparation of this manuscript.

Received for publication 28 December 1976

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