ROUGH-Smooth Dissociation of Neisseria Intracellularis

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Dissociation in the Neisseria group is infrequently reported, therefore the following occurrence may be worthy of note.

The culture was a stock strain of Neisseria intracellularis Gordon type III which had been maintained in stock by the author for about 2 years prior to the appearance of the variant. It had been cultivated for about a year on human blood agar slants and subsequently on Dorset's egg medium with frequent platings on blood agar.

The variation was first noted on a plate of human blood agar containing 0.5 per cent glucose after 24 hours' incubation at 37 C and 24 hours at about 29 C. The variation appeared to be of the simple R-S type. About 50 per cent of the colonies were typical meningococcus colonies and about 50 per cent were of the variant type. The R or variant colonies were about half the size of most of the typical S colonies. The surface of the R colonies was warty and the margins were irregular. These colonies had a heaped-up appearance and were pinkish or yellowish pink in color, in marked contrast to the typical or smooth colony. When the R colony was picked off the medium, the entire structure came away intact. It was found to be extremely hard and could only be broken up by being ground between two glass slides.

Transfers of the two colony types to blood agar plates of the same composition gave the following results: The typical S type colonies gave rise to pure cultures of S colonies through successive subcultures. The R or variant type gave cultures which consisted of about half typical colonies and half R colonies for five successive transplants. The smooth colonies from these plates invariably gave rise to pure cultures of typical colonies.

Transfers to plates of 10 per cent ascitic fluid agar containing 0.5 per cent glucose resulted in 100 per cent typical smooth Neisseria intracellularis colonies with either type as an inoculum. Transfers to Avery's blood broth with subsequent streaking on the ascitic fluid agar also resulted in pure cultures of the S type of colony. Laked blood agar prepared from the same blood used for the blood agar plates resulted in half R and half S colonies, provided they were inoculated with material from an R colony.

A sudden change occurred in transplants from the plates of the fifth successive passage of the R type of colony. The sixth successive passage on blood agar plates and laked blood agar plates gave a pure culture of a colony which resembled the original R, except in size. This new colony was much smaller than the original variant. At 24 hours it was microscopic in size, and at maximum develop-
ment (3 days) was about 1 mm in diameter. When these small R colonies were transferred to blood agar plates, they gave rise to pure cultures of similar colonies. When transferred to ascitic agar plates they maintained their characteristics. Growth in Avery’s blood broth which was streaked on ascitic agar also gave pure culture of the small R type. This small R type of colony was carried for 10 successive generations on ascitic agar, blood agar, and Avery’s blood broth without any indication of further change in colony morphology or any tendency to revert to the normal type of colony.

Microscopic examination of the various types of colonies showed them to consist entirely of gram-negative cocci and diplococci morphologically resembling Neisseria. Fermentation studies on the large R type could not be run because the cultures invariably reverted to the S type in the fermentation tubes. Such tests, however, showed the production of acid from glucose and maltose. The small R type, which was stable, produced a faint acidity in glucose, but none in maltose, sucrose, lactose, dextrin, inulin, or xylose.

The production of this variant was apparently due to the accidental use of human blood which contained antimeningococcus antibodies. This is substantiated by the following observations: First, the S or typical colony produced wide dense halos, sometimes one-half to three-fourths inches in diameter on plates prepared with this specimen of blood. Secondly, 2 months after the original observation, material from the same tube that had produced the dissociated colonies was plated on media of identical composition, except that the blood was
from a different donor. It yielded a pure culture of typical S type *Neisseria intracellularis* and no R colonies could be found on numerous plates. Thirdly, plasma obtained by centrifuging some of the same blood that produced the dissociation gave the following results in the agglutination tests: When the antigen was the Gordon type III strain, grown on ascitic agar, agglutination was positive in a dilution of 1:320. A satisfactory suspension of the small R colonies could not be prepared, and therefore the test was not satisfactory with this antigen. Similar antigens of other strains of *Neisseria intracellularis* all gave negative agglutination with this plasma. These strains included the other Gordon types and several strains isolated locally from cases of meningitis.

**SUMMARY**

A stable R variant of Gordon type III *Neisseria intracellularis* that has lost the ability to ferment maltose and that was apparently induced by antibodies is reported.