THE GROWTH OF *NEISSERIA GONORRHOEAE* ON UNENRICHED NUTRIENT AGAR

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THE purpose of this report is to record the growth of otherwise typical strains of *Neisseria gonorrhoeae* on a commonly used, unenriched meat-extract agar. From its first isolation by Leistikow and Löffler (Leistikow, 1882) and Bumm (1885), *N. gonorrhoeae* has been regarded as failing to grow on simple media such as nutrient agar without the addition of blood or serum. This is the teaching in current textbooks (Wilson and Miles, 1964; Cruickshank, 1965, p. 173; Davis *et al.*, 1968), and growth on nutrient agar is recommended as a test to distinguish between the gonococcus and commensal neisseriae (Cowan and Steel, 1965; Thayer and Garson, 1965).

During March 1966 a gonococcus was isolated that was otherwise typical, but showed the ability to grow on nutrient agar. The isolation was made from the cervix and urethra of a patient aged 18 yr. A Gram-stained film of cervical exudate showed numerous leucocytes, some of which contained Gram-negative diplococci morphologically identical with *N. gonorrhoeae*. Soon after isolation on heated blood agar, the two isolates were suspended in nutrient broth and inoculated on a nutrient agar plate. After incubation at 37°C in an atmosphere containing added carbon dioxide, both showed growth. The nutrient agar (Cruickshank, p. 741) contained the following ingredients (percentage w/v): sodium chloride (0.5), British Drug Houses (BDH) peptone (1), Oxoid "Lab-lemco" meat extract (1) and powdered agar (1.2). Growth occurred on nutrient agar for eight successive subcultures.

The isolates were typical Gram-negative diplococci, the oxidase test was positive and no growth occurred either at room temperature or on MacConkey's medium. When tested on serum agar slopes containing carbohydrates, the organisms produced acid from glucose, but maltose and sucrose were not fermented. The cultural characteristics were thus typical of a gonococcus. A culture on nutrient agar incubated for 2 days formed lenticular colonies with a smooth, shining surface. A 5-day culture showed circular colonies, up to 2 mm in diameter, with an opalescent centre and a translucent, flattened periphery; the colonies showed radial striations and the margin was crenated. Growth was also demonstrated on batches of nutrient agar supplied by the Prince of Wales Hospital and the Institute of Clinical Pathology and Medical Research, Sydney.

In May 1968 similar strains identified as *N. gonorrhoeae* were isolated from the cervix and urethra of a patient aged 16 yr. These were Gram-negative oxidase-positive cocci and, when tested on cystine peptone agar prepared from "Difco" dehydrated culture medium, produced acid from glucose but not maltose or sucrose. A freeze-dried culture of this isolate was sent to Dr E. Joan Stokes and to Dr A. E. Wilkinson who confirmed its identity as a strain of *N. gonorrhoeae* and demonstrated a positive reaction with fluorescent gonococcal antibody (personal communications, 1968). Growth on nutrient agar containing BDH peptone and "Lab-lemco" meat extract was demonstrated for nine successive subcultures.

Of 20 strains of *N. gonorrhoeae* subsequently isolated consecutively in this laboratory in a period of 15 wk in 1968, 13 were from the genital tract of adult female patients and 7 from newborn infants with purulent conjunctivitis. Specimens for culture were collected by direct plating on heated blood agar. Microscopy revealed Gram-negative intracellular diplococci in 5 of the 13 genital tract specimens and 6 of the 7 smears of conjunctival exudate. All isolates were morphologically typical and oxidase positive, and fermented

glucose but not maltose or sucrose. Five of the 20 isolates—4 from the genital tract and one from a case of conjunctivitis—when subcultured on nutrient agar soon after isolation showed growth for at least 7 subcultures.

SUMMARY

Otherwise typical strains of *Neisseria gonorrhoeae* showed growth on an unenriched nutrient agar, prepared from commonly available ingredients, when subcultured soon after isolation. Growth on nutrient agar is therefore not a valid reason for considering that a neisseria belongs to one of the non-pathogenic species.

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