THE IDENTIFICATION OF NEISSERIA GONORRHOEAE BY MEANS OF BACTERIAL VARIATION AND THE DETECTION OF SMALL COLONY FORMS IN CLINICAL MATERIAL

HARRY E. MORTON AND JOAN SHOEMAKER

Department of Bacteriology, School of Medicine, University of Pennsylvania, Philadelphia 4, Pennsylvania

Received for publication July 19, 1945

Small colony variants of *Neisseria gonorrhoeae* have been found in 3 out of 135 cases of gonococcal infections in females. These small colony variants were identified as *N. gonorrhoeae* by dissociation into the conventional colony form.

Specimens were collected on swabs from the urethra, cervix, and vagina on May 2, 1945, from case no. 246. The swabs were placed immediately in tubes containing 1 per cent bacto proteose peptone no. 3 and 0.5 per cent sodium chloride and transported to the laboratory. Within 5 minutes after arrival at the laboratory the swabs were twirled in the peptone water to make a uniform suspension, and then the moist swabs were streaked over portions of three plates of bacto proteose no. 3 "chocolate" agar containing 1 per cent bacto-supplement-A (Morton and Leberman, 1944). The remaining portions of the 3 plates were streaked with a sterile platinum loop, the plates were then placed in a glass jar, carbon dioxide added to about 10 per cent, and the jar and plates were then placed in the incubator at about 36 C for 48 hours.

When the plates were examined, the culture from the urethra contained no colonies of *N. gonorrhoeae*. The plates inoculated with the specimens from the cervix and vagina showed numerous colonies of gram-negative diplococci, which were positive to the oxidase test, using 1 per cent solution of *p*-amino-dimethylaniline monohydrochloride (Eastman Kodak Co.). The colonies were small for gonococcal colonies, being only about 0.1 to 0.2 mm in diameter. The colonies were low convex and translucent. They were isolated in pure culture on extract agar slants containing 5 per cent defibrinated horse blood and 0.1 per cent glucose and on plates of bacto proteose no. 3 "chocolate" agar containing bacto-supplement-A. The pure cultures were inoculated repeatedly into tubes of bacto phenol red broth base containing 0.25 per cent agar and 1 per cent glucose, maltose, and sucrose, respectively. The growth in the fermentation tubes was very scanty, and there was no indication of acid production in the tubes containing glucose even though gram-negative diplococci were demonstrated to be present.

The smallness of the colonies intrigued us very much because one of us has summarized the instances in which small colony forms of various pathogenic organisms had been reported in the literature (Morton, 1940). Raven (1934) reported small colony forms in her dissociative studies of *N. gonorrhoeae*, but our observations appear to be the first instances of small colonies of *N. gonorrhoeae* from clinical material.

Only by further study of these small colonies was it possible to identify the strain as an authentic gonococcus. About every third or fourth day the culture was transferred to a fresh plate of bacto proteose no. 3 "chocolate" agar with bacto-supplement-A and incubated at 36 C in an atmosphere of 10 per cent carbon dioxide. On the plate streaked on May 19 there appeared among the hundreds of small colonies about 300 colonies which were about 1 mm in diameter. These colonies were five to ten times larger than the small colonies. They were picked off to another plate of the special "chocolate" medium on May 21, and after 48 hours' incubation they appeared to be growing in pure culture, being about 2 mm in diameter. The large colonies gave a positive oxidase reaction, as did the smaller colonies, and were composed of gram-negative diplococci. Enough growth of the large colonies could be scraped readily from the plate to make a suspension for the rapid agglutination test (Phair, Smith, and Root, 1943; Noble, 1927) with chicken antigonococcal agglutinating serum (obtainable from Sharp & Dohme, Inc., Philadelphia, Pa.). A strongly positive agglutination reaction was obtained in the presence of the antigonococcal serum, and no agglutination took place in the saline control. The large colony growth was subcultured to the usual fermentation tubes and in 24 hours acid was produced from glucose but not from maltose and sucrose.

From another case (no. 194), specimens taken on April 25 yielded both small and large colony types. After a few generations on artificial medium the small colony forms dissociated into the large colony form. Not much attention was paid to the small colony forms in this case as the diagnosis of gonorrhea had been made by the identification of the large colony form as *N. gonorrhoeae*. Small colonies suspected to be *N. gonorrhoeae* were found along with large colony forms in still another case (no. 159).

The small colony forms were encountered in at least 3 out of 135 cases diagnosed as positive for the gonococcus. Schnitzer, Camagni, and Buck (1943) reported the production of small colony variants of one strain of *Staphylococcus albus* and Youmans, Williston, and Simon (1945) described the production of small colonies from 9 of 10 strains of *Staphylococcus aureus* by the action of penicillin. It was impossible to find out whether the three gonorrhoeal patients in question had been receiving any medication, either local or systemic, for suppressing gonococcal infection.

Having to depend upon the dissociation of the organisms from the small colony to the larger colony form before the organisms could be identified as *N. gonorrhoeae* in case no. 246 parallels, in some respects, the experience of Mandia, Weaver, and Scherago (1942). These investigators found dissociation necessary in order to identify a mucoid streptococcus.

REFERENCES

MANDIA, J., WEAVER, R. H., AND SCHERAGO, M. 1942 The use of dissociation in the identification of a mucoid streptococcus from a trickling filter as *Streptococcus salvarius*. J. Bact., **43**, 59.

MORTON, H. E. 1940 *Corynebacterium diphtheriae*. A correlation of recorded variations within the species. Bact. Revs., **4**, 177–226.

MORTON, H. E., AND LEBERMAN, P. R. 1944 Cultivation of the gonococcus. Advantages of chocolate agar with bacto-supplement-A as culture medium. U. S. Naval Med. Bull., **43**, 409–419.

NOBLE, A. 1927 A rapid method for the macroscopic agglutination test. J. Bact., **14**, 287–300.

PHAIR, J. J., SMITH, D. G., AND ROOT, C. M. 1943 Use of chicken serum in the species and type identification of *Neisseria*. Proc. Soc. Exptl. Biol. Med., **52**, 72–73.

RAVEN, C. 1934 Dissociation of the gonococcus. J. Infectious Diseases, **55**, 328–339.

SCHNITZER, R. J., CAMAGNI, L. J., AND BUCK, M. 1943 Resistance of small colony variants (G-forms) of a staphylococcus towards the bacteriostatic activity of penicillin. Proc. Soc. Exptl. Biol. Med., **53**, 75–78.

YOUMANS, G. P., WILLISTON, E. H., AND SIMON, M. 1945 Production of small colony variants of *Staphylococcus aureus* by the action of penicillin. Proc. Soc. Exptl. Biol. Med., **58**, 56–57.