A simple manganous chloride and Congo red disc method for differentiating *Neisseria gonorrhoeae* from *Neisseria meningitidis*

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SUMMARY Manganous chloride and Congo red incorporated into blotting paper discs have been used to differentiate gonococci from meningococci. The new technique is simple and reliable; the materials for the test are inexpensive. The method will increase the efficiency of distinguishing between the pathogenic *Neisseria* in any clinical bacteriology laboratory and especially in those in the tropical areas.

Clinically, the most important *Neisseria* are the gonococcus and the meningococcus. Gonorrhoea and other gonococcal infections are now the most common bacterial communicable diseases in the world. Meningococcal infections, although less widespread, cause pyogenic meningitis and meningococcal septicaemia, serious and often fatal conditions in many countries, especially in tropical areas. In recent times, gonococci have been isolated with increasing frequency from extragenital sites, such as the pharynx, and the clinical problems are further complicated by the isolation of meningococci from the genital tract (Catlin, 1973). Carbohydrate fermentation tests are commonly used to distinguish between these two pathogenic *Neisseria*, for the gonococcus produces acid from glucose only while the meningococcus produces acid from glucose and maltose. However, the results of fermentation patterns are not always easy to interpret and may fail to provide a clear identification in the routine clinical laboratories because of the failure of some strains of meningococci to produce detectable acid from maltose (Catlin, 1973). Although the immunofluorescence technique using specific antiserum has improved the means of differentiating gonococci from meningococci, there are still cross-reactions between the two *Neisseria* (Reyn, 1965). Cox *et al.* (1977) recently described a radiometric technique to distinguish the two pathogenic *Neisseria*. This procedure is expensive, potentially hazardous, and beyond the reach of simple clinical bacteriology laboratories.

In the course of our study on the effects of various cations on gonococci *in vitro* we noticed that whereas manganous chloride inhibited gonococci, even at a concentration of 10 μ M, a meningococcus included among a series of controls was not inhibited even at a concentration of 10 000 μ M. This observation was investigated further as it seemed that it might provide a new, simple, and inexpensive test to distinguish gonococci from meningococci in any clinical bacteriology laboratory. In a preliminary survey of strains of gonococci, we found three strains that were relatively resistant to manganous chloride. In order to provide a more satisfactory method, we examined further compounds that show a differential toxicity for gonococci and meningococci, such as Congo red, recently found effective as a means of identifying penicillin-resistant, non-penicillinase-producing gonococci (Payne and Finkelstein, 1977), ethylhydrocuprein hydrochloride (optochin), and bacitracin. We report here the results obtained with manganous chloride and Congo red mixture which was found most satisfactory for distinguishing gonococci from meningococci.

Material and methods

PREPARATION OF TEST DISCS

Manganous chloride (AnalaR), 0.5 g, is dissolved in one litre of distilled water. Congo red (Koch-Light Labs Ltd), 10 g, is added to this solution (giving final concentrations of manganous chloride 0.05% w/v and Congo red 1% w/v).

Whatman AA discs (diameter 13 mm) are then immersed in the solution, 7 ml of the solution being

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sufficient to treat 50 discs. The wet impregnated discs are sterilised in a hot air oven at 160°C for 30 minutes and subsequently may be stored in sterile universal containers at 4°C.

CULTURES

One hundred and six confirmed strains of *Neisseria gonorrhoeae* were tested, including 100 strains freshly isolated from patients attending the Special Clinic, Royal Infirmary, Sheffield and six known beta-lactamase producing strains (five strains supplied by Dr A. E. Jephcott and one strain supplied by Dr Turner, Public Health Laboratory, Liverpool). These were compared with 122 strains of meningococci representing mainly serogroups A, B, and C, supplied by Drs Abbott (Manchester), Fallon (Glasgow), and Young (Edinburgh). All cultures were first grown on Difco GC medium plus defined supplement (White and Kellogg, 1965) and checked for purity. Such fresh 18-24 hours cultures were then used to prepare a standard suspension in phosphate-buffered saline (after Brown's tube No. 1). For simplicity, a correct suspension can be obtained by emulsifying about 10 colonies of gonococci or meningococci in 2 ml of phosphate-buffered saline; this produces a minimal turbidity. A 2-mm loopful of such bacterial suspensions was then inoculated onto each quarter of a 9-cm plate of the same medium. The inoculum size is important. It is essential to have

uniformly distributed and separated bacterial colonies because as in antibiotic testing large inocula producing confluent growth may make a sensitive organism appear resistant. The dry and sterile manganous chloride and Congo red discs were then placed in the centre of each inoculated quarter of the medium. The plates were incubated for 20 hours at 36°C in a humidified atmosphere of air and 10% carbon dioxide. Cultures showing a definite zone of inhibition around the edge of the discs were recorded as sensitive and identified as gonococci while those growing to the edge of the discs were recorded as resistant and identified as meningococci.

Results

The Figure shows a control strain of *N. meningitidis* resistant to the manganous chloride and Congo red disc and three strains of *N. gonorrhoeae* including F62, an international strain, R1, a beta-lactamase producing strain, and AN97, a local strain, which is a penicillin-resistant, non-penicillinase producer; minimal inhibitory concentration (MIC) 1 mg/l with zones of inhibition around the discs. All the 106 strains of gonococci tested produced distinct zones of inhibition. Of 122 strains of meningococci tested, 121 were resistant to the manganous chloride and Congo red discs. The only sensitive strain of meningococcus was the NCTC 8554, an old laboratory

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strain. The long storage in the laboratory could have been responsible for the sensitivity towards manganese; the remaining strains provide a more representative group of currently occurring strains for the United Kingdom and Nigeria. The maltose negative strains NCTC 8339 and N1032 (Dr Abbott) were correctly identified, as were three slow glucose fermenting strains 76/12561, 77/02697, and 77/19275 (Dr Fallon).

Discussion

Browning (1931), reviewing the metal-salt therapy, quoted the experimental results of Walbum, reported in 1921, in which manganese chloride was used successfully to treat mice injected with either tetanus spores, dysentery bacilli, or staphylococci. Although we know of no specific information about the differential toxicity of manganese salts for gonococci and meningococci, Firshein and Zimmerman (1964) found that manganese could depress the nucleic acid and protein syntheses of virulent strains of group A β -haemolytic streptococci while only nucleic acid synthesis was depressed in avirulent strains. Payne and Finkelstein (1977) suggested that Congo red might be acting on the gonococcal cell envelope. More fundamental research into the role of manganese, other trace elements, and dyes on the metabolism of pathogenic *Neisseria* may further increase our understanding of the pathogenicity mechanisms in these organisms.

The pathogenic *Neisseria* are easily distinguished from the other non-pathogenic members of the group by cultural and biological characteristics (for example, growth at 22°C on unenriched medium will rule out pathogenic *Neisseria* (Reyn, 1965)). The manganous chloride and Congo red disc method described here is simple and inexpensive, and it could help in the more precise differentiation of gonococci from meningococci even in sparsely equipped clinical bacteriology laboratories in remote areas.

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