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Wheat-germ agglutination of Neisseria gonorrhoeae

A laboratory investigation

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SUMMARY Wheat-germ agglutination (WGA) was used to identify 168 strains of *Neisseria gonorrhoeae* and 105 strains of other *Neisseria* species in a routine laboratory. About one-third of the meningococci reacted with the lectin and titres with some organisms varied on repeat testing. The technique is regarded as unreliable for the identification of *Neisseria* species.

Introduction

The differentiation of *Neisseria meningitidis* and *Neisseria gonorrhoeae* has traditionally been achieved by biochemical methods and this is still the method advocated by standard textbooks.[1 2] Because of the exacting nutritional requirements of the gonococcus many attempts to improve the basal medium for carbohydrate studies[3-5] have been made. Furthermore, immunofluorescence[6] and coagglutination methods[7] have been introduced as alternatives to biochemical identification. Schaefer, Keller, and Doyle[8] have developed a new method using wheat germ agglutinin (WGA) to identify *N gonorrhoeae*. We have evaluated this lectin technique in a routine laboratory.

Materials and methods

ORGANISMS

A total of 273 strains was tested. All of the 186 strains of *N gonorrhoeae* were isolated within the past year from specimens submitted to this laboratory. The identity of each culture was established by the fluorescent antibody technique and by either sugar reactions or the coagglutination test. Strains which failed to agglutinate with WGA were tested by all three methods.

Of the 96 meningococcal isolates tested, 91 were from clinical sources (obtained from Dr J D Abbott of the Public Health Laboratory, Withington Hospital, Manchester) and had been referred for serogrouping. The two group D strains were obtained from the National Collection of Type Cultures and the remaining strains were isolated in our own laboratory. All meningococci which agglutinated with WGA were also tested with fluorescent antigonococcal conjugate and the coagglutination test.

The remaining species were identified biochemically.

MEDIA

Initially lectin agglutination was attempted on 20 cultures grown on heated blood (chocolate) agar, White and Kellogg's medium,[9] layered brain-heart infusion/blood agar, and the lysed blood Columbia agar routinely used in this department. The layered and lysed blood agars contained vancomycin and colistin; the lysed blood agar also contained trimethoprim. Cultures were tested after 24, 48, and 72 hours' growth in an atmosphere of 7% CO_2. There was good agglutination from all media after 24 hours and from all except chocolate agar after 48 hours. An approximately twofold fall in the mean titre of the 20 strains was observed after 48 hours on chocolate agar and after 72 hours on the other media. Subsequent cultures were tested from growth on lysed blood agar after 24 or 48 hours' or from chocolate agar after 24 hours' incubation.

WHEAT-GERM AGGLUTININ

This was obtained from Sigma Chemical Co, Fancy Road, Poole, Dorset, and EY Laboratories (UK agents, TCS Ltd, 10-12 Henry Road, Slough, Berks). No difference was noted in the reactivity of preparations from either supplier. Stock solutions were prepared in phosphate-buffered saline (PBS; 0·05 mol/l disodium phosphate, 0·15 mol/l sodium chloride, pH 7·2) at a concentration of 1 mg/ml. Aliquots of this solution were distributed in plastic vials and frozen at −20°C. For use, dilutions were

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made in PBS. The lectin is stable and no deterioration was detected after storage of a 1/16 dilution at 4°C for three weeks.

AGGLUTINATION

Suspensions were made by removing growth from the culture plate with a cottonwool swab and emulsifying it in 0·8 ml PBS containing formalin at a final concentration of 0·5% (v/v). The density of the suspension was adjusted to an absorbance of 0·5-0·75 (10 mm path length) at 595 nm. Doubling dilutions of the stock WGA solution from 1/2 (500 μg/ml) to 1/512 (2 μg/ml) were prepared in PBS. One drop of each dilution was placed on a flat glass tile with ceramic rings (18 mm in diameter) together with a PBS control. To each drop was added one drop of the organism suspension. The tile was rotated in a horizontal plane at 100 rev/min for 10 minutes. The tests were read macroscopically and agglutination was graded as: + + + +, very few clumps in clear supernatant; + + +, medium-sized clumps, clear supernatant; + +, small easily seen agglutinates; +, agglutination just visible to the naked eye; and ±, fine agglutinates, visible only with a hand lens. The highest WGA dilution giving + + agglutination was taken as the end point. A stock strain of known reactivity (WGA titre 1/64) was included with each batch of tests to check the activity of the lectin. Strains of *N gonorrhoeae* failing to agglutinate and *N meningitidis* agglutinating at 500 μg/ml were retested on a separate occasion.

Results

The results of titrations of WGA against 269 strains of *Neisseria* spp and four strains of *Branhamella catarrhalis* are given in table I. A concentration of 62 μg/ml was chosen as the breakpoint, which is the same as that of Schaefer, Keller, and Doyle.[8] Only nine of 168 strains of gonococci failed to agglutinate with WGA at this concentration at the first attempt.

TABLE I *Agglutination of Neisseria species with wheat-germ lectin (WGA) at a concentration of 62 μg/ml*

Species	No of strains tested	WGA result +	WGA result −	Autoagglutinable
N gonorrhoeae	168	159	9*	0
N meningitidis	96	21†	75‡	0
N lactamica	2	1	1	0
N subflava	1	0	0	1
N flavescens	2	0	0	2
Br catarrhalis	4	0	0	4

+ Positive; − negative
*Five of these strains gave positive results on repeat testing
†Three strains gave negative results on repeat testing
‡Four strains gave positive results on repeat testing

On repeat testing, five of these strains agglutinated at concentrations of 62 μg/ml or less, while the other four showed no agglutination. Some 25% of strains agglutinated with concentrations as low as 2 μg/ml.

Of the 96 strains of *N meningitidis*, 21 were agglutinated at 62 μg/ml at the first attempt. On repeat testing, three of these no longer showed agglutination, and four strains which had previously given a negative result showed agglutination. The pattern of agglutination with meningococci is given in further detail in table II. Four of the five WGA-negative non-groupable strains agglutinated at concentrations above 62 μg/ml and on repeat testing gave a positive result, agglutinating at 62 μg/ml or less. The fifth strain failed to agglutinate at 500 μg/ml on both occasions.

The two strains of *N lactamica* tested agglutinated at 125 and 4 μg/ml. All the commensal species were autoagglutinable.

TABLE II *Agglutination of serogroups of N meningitidis with WGA (62 μg/ml)*

Serogroup	No of strains tested	WGA result +	WGA result −	Source of WGA-positive strains
A	4	0	4	
B	37	3	34	Throat (3)
C	12	3*	9	Blood (1), CSF (1), Throat (1)
D	2	0	2	
W 135	7	1	6	Vagina
X	1	1	0	Throat
Y	6	1†	5	Throat
29 E/Z	10	0	10	
Not groupable	17	12	5‡	Throat (11), urethra (1)
Total	96	21	75	

+ Positive; − negative
*Two strains became negative on repeat testing
†One strain became negative on repeat testing
‡Four strains became positive on repeat testing

Discussion

Identification of species of *Neisseria* is important to the clinician and to the epidemiologist but often presents difficulties to the bacteriologist. The oldest method of differentiation depends on the varying abilities of different species to attack carbohydrates. More recent methods depend on determining the reaction of cultures with specific antisera. Occasional strains of *N meningitidis*, however, are encountered which do not attack maltose,[10] and most meningococci when reacted with an antigonococcal conjugate produce a low-grade fluorescence, which may be construed as a positive result by an inexperienced worker. The coagglutination test overcomes these problems but is expensive in the commercial kit form (£0·82 per test).

The lectin method of Schaefer *et al*[8] offered a cheaper solution to the problem. At a concentration of 62 μg/ml they found that wheat-germ lectin agglutinated over 99% of the 165 gonococcal strains tested, whereas only one strain of *N meningitidis* out of 24 was agglutinated. In their study only one non-groupable strain of *N meningitidis* was tested. In our study 16 out of 17 non-groupable strains gave a positive reaction on at least one occasion.

Our findings support those of Frasch,[11] who found that 50% of non-groupable meningococci agglutinated with WGA at 62 μg/ml. Our practice of testing against dilutions of WGA from 500 μg/ml to 2 μg/ml, instead of against a single concentration, showed some minimally reactive strains of both gonococci and meningococci which on repeat testing agglutinated at 62 μg/ml or less. These variations in titre may reflect quantitative variations in the capsule of the organism or a change in the ratio of non-capsulated forms in the cultures when tested again after storage in glycerol broth at −70°C.

The composition of the culture medium and the age of the cultures may also have some effect, since titres fell more rapidly with organisms grown on chocolate agar than with those grown on other media. The positive findings with meningococci other than the non-groupable strains may be explained by Frasch's finding that selection of non-encapsulated strains for testing with WGA resulted in agglutination. Only one of these groupable strains which agglutinated with WGA, however, had become non-groupable on repeat testing (personal communication, 1980). Reversion to a negative reaction on repeat testing of three strains was the result of a fall in titre to 125 μg/ml with two strains and a failure to agglutinate at 500 μg/ml in one strain.

It is now established[11] that the lectin agglutinates many strains of non-groupable meningococci and this makes it unsuitable for use in identifying *Neisseria* spp from pharyngeal sources. Variations in titre of both meningococci and gonococci also make selection of a single concentration of lectin as a criterion of positivity difficult. These problems suggest that the technique may be unreliable for the identification of *N gonorrhoeae* even if its use was restricted to isolates from genital sources, where the probability of isolating meningococci is small.[12 13]

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