SLIDE AGGLUTINATION TECHNIQUE FOR THE RAPID DIFFERENTIATION OF *MIMA POLYMORPHA* AND HERELLEA FROM THE NEISSERIAE

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The genera *Mima* and *Herellea*, members of the tribe *Mimeae* (De Bord, 1942), contain organisms which morphologically resemble the *Neisseriae* upon primary isolation. Since they are being isolated in increasing numbers from the spinal fluid of patients with acute meningitis, a method for their differentiation from the *Neisseriae* would be advantageous.

Schaub and Hauber (1948) classified a gram-negative rod, of which the diplococcal forms predominated on solid media, as *Bacterium anitratum*, or B5W. *B. anitratum* has also been isolated from cases of meningitis. Ewing (1949) stated that on the basis of morphological characteristics and physiological reactions, *B. anitratum* should be classified in the tribe *Mimeae*. Hence, we have also included in our studies the 10 capsular serotypes of *B. anitratum* designated by Ferguson and Roberts (1950). Stuart *et al.* (1949) found that heated cells of *B. anitratum* were inagglutinable. Ferguson and Roberts (1950) observed, in serological studies with a limited number out of 109 strains of *B. anitratum*, that of several antigens prepared from a single strain and heated at 121 C for up to 3 hr, some agglutinated with the homologous antiserum while others remained inagglutinable. We have also made this observation with strains of *Mima polymorpha* and *Herellea* sp. collected here. In addition, we have found that living suspensions of these organisms are inagglutinable in homologous antisera. In a previous publication, typing of *M. polymorpha* by a precipitin technique has been reported (Cary *et al.*, 1956). The precipitating antigen described therein behaved as a specific serotype rather than reacting as a group factor with all *Mimeae* antisera. For this reason, we initiated experiments to determine whether a common group antigen was present and, if so, whether its detection would facilitate rapid differentiation of strains of *M. polymorpha* from the *Neisseriae*. In this laboratory, various physical and chemical agents were used to treat strains of *M. polymorpha* and *Herellea* sp. in order to obtain a suspension of organisms which could be used in serological procedures. These included treatment with acid, alkali, formamide, and pangestin, a mixture of pancreatic enzymes. Of the methods tried, it was found that by treatment of cells with alkali the presence of a group antigen for *M. polymorpha* and one for *Herellea* sp. could be demonstrated.

MATERIALS AND METHODS

Organisms. Strains representing the 10 serotypes of *B. anitratum* (Ferguson and Roberts, 1950) were obtained through the kindness of Dr. Ferguson. The strains of *M. polymorpha* and *Herellea* sp. used in these studies were isolated from spinal fluid, pleural fluid, blood, urine, sputum, and soft tissues abscesses.

Antisera. Immune sera were prepared by the inoculation of rabbits by several intravenous injections with washed formalized vaccines over a period of 3 weeks.

Adsorption. Polyvalent *M. polymorpha* antiserum was prepared using equal amounts of immune sera prepared from strains 24 and 44, since of the 9 antisera prepared, only these 2 agglutinated all the strains of *M. polymorpha*. As these sera cross agglutinated only with strains of *B. anitratum* which have been shown to be morphologically and physiologically related to the Mimeae, no adsorption was necessary. A polyvalent Herellea antiserum was prepared by combining equal amounts of sera for strains 1856, 1847 and 499, which sera together agglutinated all strains of Herellea. This unadsorbed pooled serum cross reacted with 2 strains of *M. polymorpha* and with one of the Alkalescens-Dispar group, 01. Adsorption with each of these strains using 0.1 ml of wet, packed, living cells per ml of antiserum at 50 C for 1 hr removed the common agglutinins.

Antigens. Using a modification of the alkali extraction procedure of Landy *et al.* (1955) for preparing soluble antigens for hemagglutination tests, it proved possible to prepare a bacterial antigen for slide agglutinations. A suspension equivalent to 2 billion organisms per ml was made from 18 hr cultures on chocolate agar plates in 1 ml of freshly prepared 0.05 N sodium hydroxide in distilled water. Chocolate agar was used since it is a common medium for spinal fluid cultures. Blood agar, infusion agar and trypticase soy agar function equally well in antigen preparation. The bacterial suspension was placed in a boiling water bath for 10 min, cooled and centrifuged. The cells were resuspended in 1 ml of saline for slide agglutination tests. The alkaline supernatants were saved for precipitin reactions.

Agglutination tests. One drop of polyvalent antiserum was placed on a ruled glass slide and one drop of antigen added. Slides were rocked for 3 min and read.

Precipitin tests. The alkaline extracts were adjusted to pH 7.0 and used as antigens for typing strains of *M. polymorpha*. Precipitin tests were performed according to the procedure of Lancefield (1933). Unadsorbed immune sera were used in a final dilution of 1:2 and read after 10 min at room temperature.

RESULTS AND DISCUSSION

An effective polyvalent grouping antiserum for *M. polymorpha* was prepared by combining antisera for 2 strains of this organism. Similarly, a polyvalent Herellea antiserum was obtained by combining sera for 3 strains. The basis for selection of these sera is presented in table 1. It was found that of the 9 antisera for *M. polymorpha*, strains 24 and 44, each agglutinated 19 of the 22 test strains and, when combined, all test strains reacted. Thus, a diagnostic grouping serum resulted from pooling equal volumes of these sera. Similarly, with Herellea, three strains yielded antisera which agglutinated respectively 15 to 18 different test strains. When combined in equal volumes these sera constituted an adequate grouping serum, in which all Herellea encountered reacted.

A total of 42 strains of *M. polymorpha* and 10 of the serologically analogous *B. anitratum* reacted in the *M. polymorpha* grouping serum. Of these, 34 strains were typable by specific sera as well, while the remaining 18 strains were not. The latter are being investigated for the possible presence of additional serotypes.

TABLE 1

Slide agglutination reactions with alkali treated cells

Organism	Total No. Strains Tested	*Mima polymorpha* Antisera and No. of Strains Reacting									
		44+24	24	44	23	21	2902	803	1609	1120	979
M. polymorpha	22	22	19	19	11	9	3	5	11	9	3
		Herellea Antisera and No. of Strains Reacting									
		499+1856+1847		499	1847	1856	2520	1836	1881		
Herellea sp.	22	22		16	18	15	9	10	9		

TABLE 2

Slide agglutination tests with Mima polymorpha and adsorbed Herellea sp. antisera

Antigens	Polyvalent *M. polymorpha* Antiserum	Polyvalent Adsorbed Herellea Antiserum	No. of Strains Tested
Mima polymorpha	+	−	42
Bacterium anitratum	+	−	10
Herellea sp.	−	+	38
Salmonella sp. Group A–E	−	−	10
Shigella sp. Group A–D	−	−	10
Alkalescens-Dispar Group 01–04	−	−	4
Escherichia coli	−	−	9
Klebsiella sp. (5-0 Groups)	−	−	5
Neisseria meningitidis	−	−	6
Neisseria sicca	−	−	1
Neisseria catarrhalis	−	−	1
Haemophilus sp.	−	−	2
Pasteurella pestis TJW	−	−	1
Alcaligens faecalis	−	−	2
Pseudomonas aeruginosa	−	−	4
Achromobacter sp.	−	−	4

The possibility of cross reactions between *M. polymorpha* and Herellea grouping sera and various gram-negative organisms was explored as summarized in table 2. It will be seen that the

polyvalent *M. polymorpha* and adsorbed polyvalent Herellea antisera were specific for the respective groups. No cross reactions were obtained with any of the 51 gram-negative bacilli nor with 8 strains of 3 species of Neisseria tested.

We have found that strains must be in a smooth, mucoid form in order to demonstrate the type specific antigen and group antigen. When the stock cultures were maintained in semisolid agar at room temperature, antigens reactive for the systems employed were lost after several transfers. However, serial transfer on trypticase soy agar containing 5 per cent glycerin has been found to restore a mucoid colony form which is antigenically active.

The alkaline supernatants from the treated cells, when adjusted to pH 7.0, can be used for typing of strains of *M. polymorpha* by the precipitin technique as previously described (Cary *et al.*, 1956). Preliminary trials indicated that these extracts were type specific as are the extracts prepared using the procedure of Maxted (1948).

With this alkali extraction procedure, it is now possible to differentiate members of the genus *Neisseria* from *Mima polymorpha* and *Herellea* sp. serologically upon primary isolation. In 14 to 18 hr the potential causative agent in cases of meningitis can be more effectively characterized.

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SUMMARY

Antigens for slide agglutination have been prepared by alkali treatment of cells of *Mima polymorpha* and *Herellea* sp. It was found that 2 group antigens occur in *M. polymorpha* while 3 were recognized in *Herellea* sp. Two strains of *M. polymorpha* and 1 of the Alkalescens-Dispar group cross reacted with unadsorbed polyvalent Herellea antisera. These reactions were eliminated by adsorption. By combining appropriate antisera, pooled grouping sera were prepared for *M. polymorpha* and for *Herellea* sp. *M. polymorpha* and *Herellea* sp. can thus be differentiated from the members of the genus *Neisseria* upon primary isolation. The adsorbed Herellea polyvalent antiserum and *M. polymorpha* polyvalent antiserum tested against the 59 strains of other bacteria listed in table 2 showed no cross agglutination.

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