

## TYPING OF *MIMA POLYMORPHA* BY A PRECIPITIN TECHNIQUE

SYLVIA G. CARY, ROBERT B. LINDBERG, AND JOHN E. FABER, JR.

*Departments of Bacteriology, Walter Reed Army Institute of Research, Washington, D. C., and University of Maryland, College Park, Maryland*

Received for publication February 16, 1956

The members of the tribe *Mimeae* constitute a diagnostic and clinical bacteriological problem since they resemble other gram negative bacteria and are frequently not recognized in the laboratory. Morphologically they resemble the *Neisseriae* since diplococcal forms predominate on solid media. Deacon (1945) reported the isolation of *Mima polymorpha* from 8 cases of meningitis, and Ewing (1949) published similar results based on cases in Italy in 1942. Strains isolated from spinal fluid cultures have been received at the Walter Reed Army Institute of Research during the past 5 years. To facilitate more rapid recognition and differentiation of these organisms from the *Neisseriae* in cases of meningitis, Maxted's technique (1948), using a proteolytic enzyme for the preparation of precipitating antigens for serological typing, has been applied.

### MATERIALS AND METHODS

**Organisms.** Strains representing 10 serologically distinct serotypes (Ferguson and Roberts, 1950) were obtained through the kindness of Dr. Ferguson. These had been isolated from spinal fluid, pleural fluid, blood, sputum, and urine. Fourteen strains collected in this laboratory included nine from spinal fluid; one each from pleural fluid, blood, and sputum; and two from unknown sources. Six strains of *Neisseria meningitidis*, including Types I, II, and IIa, and one each of *Neisseria sicca* and *Neisseria catarrhalis* were included for comparison.

**Proteolytic filtrate.** A modification of the procedure of Maxted (1948) employing a strain of *Streptomyces albus* cultivated on semi-solid agar was used as the source of the proteolytic enzyme. The basal medium consisted of 0.5 per cent proteose peptone (Armour), 0.3 per cent yeast extract, 0.2 per cent  $\text{Na}_2\text{HPO}_4$ , 0.2 per cent glucose, and 0.6 per cent agar. The medium was distributed in Kolle flasks and autoclaved at 121 C for 20 min. Adjustment of the pH was not necessary since slightly acid medium is required for maximum production of the enzyme. After

autoclaving, the medium was cooled to 50 C and 2 per cent Fildes' extract (1920) added. This extract consists of a peptic digest of rabbit red blood cells, adjusted to a pH of 7.6. Growth from Sabouraud's agar slant cultures of *S. albus* grown at 37 C for 6 days was used as inoculum for the mass culture. After 6 days' incubation at 37 C, the Kolle flasks were frozen for 24 hr and then thawed at room temp. The fluid from the disrupted agar containing the enzyme was adjusted to pH 7.5 and filtered through a sintered glass filter, bottled, and stored at 5 C. To avoid a drop in potency the filtrate should be lyophilized if it is to be stored more than 4 months.

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

**Precipitin tests.** The test strains of *M. polymorpha* and other organisms were grown on chocolate agar and on trypticase soy agar plates for 18 hr at 37 C. These media were selected because in this laboratory they are used routinely in the primary isolation of *N. meningitidis*, and *M. polymorpha* must be distinguished from the *Neisseriae* in cases of meningitis. A 3-mm loopful of cells from the agar culture was placed in 0.25 ml of the proteolytic filtrate in a 50 C water bath for 30 min or until the fluid cleared to a point indicating that substantially all the cells were lysed. The resulting fluid constituted the antigen for testing. 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 temp.

**Adsorption of sera.** Eighteen-hour living cultures were used as adsorbing antigens. Two-tenths of a milliliter of cells, centrifuged at 2500 rpm for 30 min, was added to each ml of indicated antiserum, incubated at 50 C for 1 hr, and centrifuged. The adsorbed sera were tested undiluted and at a dilution of 1:2.

## RESULTS AND DISCUSSION

The behavior of the strains of *M. polymorpha* studied clearly indicated the presence, in the Maxted extract, of a type specific antigen but not of group-reacting antigen. The precipitin technique permitted recognition of nine new provisional serotypes, in addition to the 10 originally demonstrated by Ferguson and Roberts (1950). These serotypes were designated by numbers from 11 through 19, and the initial type strain for each was lyophilized to constitute a reference source. In the case of provisional types 12, 13, 14, and 19, two or more strains were collected. It was demonstrated by reciprocal adsorption procedures that these additional strains were specific for the types indicated. They do not contain an antigen capable of cross reacting with the type specific factor in the system described. The Maxted extracts prepared from the strains of *Neisseriae* included in the test showed no cross reactions with any of the 19 serotypes of *M. polymorpha*.

The Maxted technique (1948) as described is a rapid, accurate means of serological identification of strains of *M. polymorpha* since the antigens can be prepared directly from cultures on chocolate agar plates and the proteolytic filtrate used in the preparation can be stored at 5 C for at least 4 months, or lyophilized with no loss of potency. Thus, in cases of meningitis, within 14 to 18 hr after spinal fluid is plated out, the distinction between *M. polymorpha* and *N. meningitidis* as the potential causative agent can be established.

At the present time, additional strains of *M. polymorpha* derived from a variety of pathological conditions are being studied in order to establish the probable distribution of different serotypes. Of the 19 types studied so far, no single type seemed to be particularly associated with meningitis, since types 2, 12, and 17 have been isolated

from blood cultures, while types 1 through 10 have been isolated from urine. Ferguson and Roberts (1950) suggested that in view of the frequent occurrence of this organism in urinary infections, its pathogenicity might resemble that of certain types of *Escherichia coli*.

## ACKNOWLEDGMENT

The authors are indebted to Dr. William H. Ewing, Communicable Diseases Center, Chamblee, Georgia, and Miss Ann Hoffman, Mt. Sinai Hospital, Chicago, for additional strains of *M. polymorpha*.

## SUMMARY

By the use of an enzymatic digestion process (the Maxted procedure), the existence of at least 19 serological types of *Mima polymorpha* has been demonstrated. The antigen present is type specific rather than group specific. There were no cross reactions between the antigens prepared from the strains of *Neisseriae* studied and the designated serotypes of *M. polymorpha*.

## REFERENCES

- DEACON, W. E. 1945 A note on the tribe *Mimeae* (De Bord). *J. Bacteriol.*, **49**, 511-512.
- EWING, W. H. 1949. The relationship of *Bacterium anitratum* and members of the tribe *Mimeae* (De Bord). *J. Bacteriol.*, **57**, 659.
- FERGUSON, W. W. AND ROBERTS, LUCILLE F. 1950 A bacteriological and serological study of organism B5W (*Bacterium anitratum*). *J. Bacteriol.*, **59**, 171-183.
- FILDES, P. 1920 A new medium for the growth of *B. influenza*. *Brit. J. Exptl. Pathol.*, **1**, 2, 129.
- LANCEFIELD, R. C. 1933 Serological differentiation of human and other groups of hemolytic streptococci. *J. Exptl. Med.*, **57**, 571-595.
- MAXTED, W. R. 1948 Preparation of streptococcal extracts for Lancefield grouping. *Lancet*, **2**, 255-256.