Possible Influence of Antibiotic Therapy on Usefulness of Metabolic Inhibition Test for Diagnosis of *Mycoplasma pneumoniae* Infections

THOMAS F. SMITH AND ERNEST C. HERRMANN, JR.

Department of Microbiology and Immunology, Mayo Clinic and Mayo Foundation, Rochester, Minnesota 55901

Received for publication 11 September 1970

Certain antibiotics, if in serum, simulate specific antibody in the metabolic inhibition test for diagnosis of *Mycoplasma pneumoniae* infections. This effect is not eliminated by presence of serum or heat treatment.

Serological tests used for the diagnosis of *Mycoplasma pneumoniae* infections include immunofluorescence (4), indirect hemagglutination (12), complement fixation (1), cold agglutinins (5, 13), and metabolic inhibition (MI; 11; K. E. Jensen, Bacteriol. Proc., p. 70–71, 1964). Of these, the MI test has been used extensively since antibody to *M. pneumoniae* can be detected with high degrees of specificity (6) and sensitivity (10) by measuring the capacity of serum to prevent the reduction of 2,3,5-triphenyltetrazolium chloride (Jensen, Bacteriol. Proc., 1964) or to inhibit acid production from glucose by this organism (11).

This report shows that certain antibiotics, if present in serum as a result of antibiotic therapy, might produce erroneous results in the MI procedure.

A single broth culture of *M. pneumoniae* strain FH (L. Hayflick, Stanford Medical School) containing $2 \times 10^8$ colony-forming units (CFU)/ml was used.

Stock aqueous solutions (1 mg/ml) were prepared, according to the manufacturers' assay, for the following antibiotics: chlorotetracycline-hydrochloride (Lederle Laboratories, Pearl River, N.Y.), chloramphenicol (Parke, Davis & Co., Detroit, Mich.), dihydrostreptomycin sulfate (Calbiochem, Los Angeles, Calif.), and gentamicin sulfate (Schering Corp., Bloomfield, N.J.). Erythromycin (Eli Lilly & Co., Indianapolis, Ind.) was dissolved in a minimal volume of ethyl alcohol and diluted with water to 1 mg/ml.

Antibiotics were added to sera free of antibody to *M. pneumoniae*, as indicated by the MI test, and to unsupplemented PPLO broth (Difco Laboratories, Detroit, Mich.). A portion of each preparation was heated at 56°C for 30 min before the MI test. A similarly treated antibiotic-free serum was included as a control in each test. Serial twofold dilutions of the antibiotic-containing serum and of the broth were made in plastic microtiter trays with U-shaped cups (Lindbro Chemical Co. Inc., New Haven, Conn.) by using the medium and procedure described by Taylor-Robinson et al. (11). Pressure-sensitive film (Bioquest, Cockeysville, Md.) was applied to the tray after addition of 0.05 ml of a *M. pneumoniae* suspension containing 1,000 or 100 CFU resulting in 1 color-changing unit at 5 and 7 days, respectively (11). The final volume in each cup was 0.2 ml.

Certain antibiotics, at levels that might well occur in persons receiving therapy, produced a false-positive reaction indicating the presence of antibodies for *M. pneumoniae* (Table 1). Treatment of antibiotic-containing human sera at 56°C for 30 min failed to eliminate these effects. Also, there was no indication of serum binding of the antibiotics in this test.

Jao and Finland (3) and Slotkin et al. (9) demonstrated the inhibition of *M. pneumoniae* in vitro and in vivo by several antibiotics, especially erythromycin. Where directly applicable, the antibiotic sensitivities compare favorably with those found by Jao and Finland (3), except for the relative insensitivity of *M. pneumoniae* to gentamicin reported here. Shanes et al. (8) and Rasch and Mogabgab (7) established the efficacy of erythromycin treatment for patients with primary atypical pneumonia caused by *M. pneumoniae*. However, the clinical picture of primary atypical pneumonia is not always well defined (2), and the choice of antibiotic for use in suspected cases is also confused by conflicting reports on the merits of other antibiotics (3). It can be readily realized, therefore, that acute or convalescent phase sera might contain antibiotics and produce
false results in the MI test. This is especially true for erythromycin, perhaps the drug of choice for *M. pneumoniae* infections. The fact that the therapy of a patient is known of little help since only sera from drug-free patients would be useful. Furthermore, there can be times, due to self-medication or treatment by more than one physician, when the drugs being used are not well established. If the MI test is to be diagnostically useful, those antibiotics inhibitory to *M. pneumoniae* would have to be withheld, and this is an unlikely possibility. From these considerations, there appears to be no practical way whereby antibiotic interference in the MI test can be eliminated.

We thank Larry L. Woods for his excellent technical assistance.

**LITERATURE CITED**

1. Chanock, R. M., W. D. James, H. H. Fox, H. C. Turner, M. A. Mufson, and L. Hayflick. 1962. Growth of Eaton PPLO in broth and preparation of complement fixing antigen. Proc. Soc. Exp. Biol. Med. 110:884-889.

2. Couch, R. B. 1969. Diagnosis and treatment of *M. pneumoniae* disease in man, p. 683–695. In L. Hayflick (ed.), The Mycoplasmas and the L-phase of bacteria. Appleton-Century-Crofts, Inc., New York.

3. Jao, R. L., and M. Finland. 1967. Susceptibility of *Mycoplasma pneumoniae* to 21 antibiotics in vitro. Amer. J. Med. Sci. 253:639–650.

4. Liu, C. 1957. Studies on primary atypical pneumonia. I Localization, isolation, and cultivation of a virus in chick embryos. J. Exp. Med. 106:455–466.

5. Peterson, O. L., T. H. Ham, and M. Finland. 1943. Cold agglutinins (autohemagglutinins) in primary atypical pneumonia. Science 97:167.

6. Purcell, R. H., R. M. Chanock, and D. Taylor-Robinson. 1969. Serology of the mycoplasmas of man, p. 221–264. In L. Hayflick (ed.), The Mycoplasmas and the L-phase of bacteria. Appleton-Century-Crofts, Inc., New York.

7. Rasch, J. R., and W. J. Mogabgab. 1966. Therapeutic effect of erythromycin on *Mycoplasma pneumoniae* pneumonia. Antimicrob. Ag. Chemother.—1965, p. 693–699.

8. Shanes, J. M., R. B. George, W. B. Holliday, J. R. Rasch, and W. J. Mogabgab. 1970. Comparison of antibiotics in the treatment of mycoplasmal pneumonia. Arch. Intern. Med. 125:680–684.

9. Slotkin, R. I., W. A. Clyde, Jr., and F. W. Denny. 1967. The effect of antibiotics on *Mycoplasma pneumoniae* in vitro and *in vivo*. Amer. J. Epidemiol. 86:225–237.

10. Steinberg, P., R. J. White, S. L. Fuld, R. R. Gutekunst, R. M. Chanock, and L. B. Senterfit. 1969. Ecology of *Mycoplasma pneumoniae* infections in marine recruits at Parris Island, South Carolina. Amer. J. Epidemiol. 89:62–73.

11. Taylor-Robinson, D., R. H. Purcell, D. C. Wong, and R. M. Chanock. 1966. A colour test for the measurement of antibody to certain Mycoplasma species based upon the inhibition of acid production. J. Hyg. 64:91–104.

12. Tully, J. G. 1943. Erythrocyte-modifying capacity and serological reactivity of cell components of human Mycoplasma (PPLO) strains. Proc. Soc. Exp. Biol. Med. 114:704–709.

13. Turner, J. C. 1943. Development of cold agglutinins in atypical pneumonia. Nature (London) 151:419–420.

| Table 1. Effect of antibiotics on metabolic inhibition test |
|-----------------------------------------------------------|
| Diluent/Isoculum | Treatment/medium | Erythromycin | Dihydrostreptomycin | Chlorotetracycline | Chloramphenicol | Gentamicin |
|------------------|-----------------|--------------|---------------------|-------------------|----------------|------------|
| Human serum      | Heated          | 10³          | 0.01d               | 2.34d             | 6.25d          | 6.25d      | 9.38d      |
|                  | Unheated        | 10³          | 0.01d               | 1.17d             | 1.56           | 4.69d      | 9.38d      |
| Metabolic inhibition medium | Heated | 10³          | 0.01d               | 3.12              | 3.12           | 6.25      | 12.50      |
|                  | Unheated        | 10³          | 0.01d               | 0.39              | 1.56           | 6.25      | 12.50      |
| PPLO broth       | Heated          | 10³          | NT*                  | 1.56              | 6.25           | 3.12      | 6.25      |
|                  | Unheated        | 10³          | NT                  | 0.78              | NT             | 6.25      | 12.50      |

a Values expressed as colony-forming units.
b Values expressed as micrograms per milliliter (final concentration).
c At 56 C for 30 min.
d Average values for two or more tests; otherwise a single determination was made.
e Not tested.