If these conclusions are confirmed, we intend to use ^{36}Cl dating to refine the chronology of Searles Lake sediments in sections of the KM-3 core that are not well dated by other methods and then to date and correlate the intervals of basin interconnection, using cores and samples from Owens, China, Panamint, and Manly (Death Valley) lakes. It should then be possible to apply the technique to saline sediments in other parts of the world.

FRED M. PHILLIPS
Geoscience Department and
Geophysical Research Center, New Mexico Institute of Mining and Technology, Socorro 87801
GEORGE I. SMITH
Branch of Energy Minerals, U.S. Geological Survey, Menlo Park, California 94025
HAROLD W. BENTLEY
Department of Hydrology and Water Resources, University of Arizona, Tucson 85721
DAVID ELMORE
HARRY E. GOVE
Nuclear Structure Research Laboratory, University of Rochester, Rochester, New York 14627

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Serologic Evidence of Chlamydial and Mycoplasmal Pharyngitis in Adults

Abstract. *In a study of 763 adult patients we found serologic evidence of infection (a fourfold increase in antibodies) with* Chlamydia trachomatis *in 20.5 percent of the patients and with* Mycoplasma pneumoniae *in 10.6 percent, but with group A streptococcus (by culture) in only 9.1 percent. Pharyngitis, the most common problem for which patients seek medical care in the United States, may be caused by nonviral, potentially treatable organisms more often than had been suspected.*

Pharyngitis accounts for over 40 million visits by adults to medical facilities each year in the United States (*1*). It also accounts for over 100 million days of absence from the workplace annually, or more days than are lost from all strikes, work stoppages, and lockouts combined (*2*). In patients with a throat culture that is negative for beta-hemolytic group A streptococci, the cause of pharyngitis usually is assumed to be viral, and antibacterial treatment is withheld (*3*). We sought to determine whether two nonstreptococcal agents potentially treatable by currently available antibacterials—*Chlamydia trachomatis* and *Mycoplasma pneumoniae*—might be playing a role in this extremely common illness. We therefore conducted a prospective 1-year study of adults seen in four general medical practices in New England.

The study group comprised 763 unselected patients with either a chief complaint or an elicited complaint of sore throat. The mean age (± standard deviation) was 30.9 ± 10.5 years; 61 (8 percent) of the patients were 50 or older. From each patient a standardized battery of medical history and physical examination data were obtained. A double-swab throat culture was planted on separate, nonselective sheep blood agar plates that were stabbed according to routine methods (*4*) and incubated anaerobically to enhance beta-hemolysis. Group A streptococci were identified by beta-hemolysis and bacitracin sensitivity.

From each patient we obtained a serum specimen for measuring acute-phase antibodies. The acute-phase serum was tested for heterophil antibody by the horse-cell agglutination method or the Paul-Bunnell sheep cell agglutination method. Since we could not hope to obtain convalescent sera from all 763 patients, we created a stratified random sample that selected more heavily from patients with explicitly defined clinical findings of more severe disease, but which sampled from patients with all degrees of illness severity. A convalescent serum specimen was obtained from 166 patients approximately 6 weeks (45.6 ± 16.4 days) later (*5*). Acute- and convalescent-phase sera were tested for antibodies to numerous diverse organisms. The individuals performing the tests had no knowledge of the clinical data on the patients. Not all of the paired sera could be tested for antibodies to each organism: in some cases anticomplementary activity and other technical difficulties precluded accurate testing (*6*).

Antibodies to streptococcus were measured with the Streptozyme test: a fourfold increase was regarded as diagnostic, and is known to be closely correlated with the measurement of antistreptolysin-*O* antibody (*7*). Antibodies to influenza viruses A and B, parainfluenza viruses 1, 2, and 3, adenovirus, and respiratory syncytial virus were measured in the Vermont State Public Health Laboratory with standard complement fixation techniques (*8*): a fourfold increase was regarded as diagnostic. Complement-fixing antibodies to *M. pneumoniae* (*9*) were measured in the laboratory of R. Chanock at the National Institutes of Health: again, a fourfold increase was regarded as diagnostic. Using the simplified microimmunofluorescent method of Wang *et al.* (*10*), one of us (J.S.) measured immunoglobulin G (IgG) and immunoglobulin M (IgM) antibodies to *C. trachomatis*: a fourfold increase in these antibodies (except to the A complex serotypes) was regarded as positive. Representative *Chlamydia psittaci* isolates of avian and mammalian origin were also included as slide antigens (and the results were never positive). We did not attempt to culture *M. pneumoniae* or *C. trachomatis*.

Frequency rates of different organisms, when based on the tests of the paired sera, were estimated separately for each stratum. The rates for each stratum were determined by using a logarithmic linear model (*11*) to reduce the sampling error associated with direct estimates based on small numbers of patients in particular strata. These rate estimates were applied to unsampled members of each stratum in determining the overall frequency of the different pathogens.

The frequency of evidence of infection with the various organisms was estimated in all patients with pharyngitis, as well

Table 1. Estimated frequency of different pathogens in all adult patients with pharyngitis and in that subset of patients with pharyngitis only and no symptoms or signs of bronchopulmonary infection. In some cases the percentages shown are slightly different than those calculated from the ratios, due to the use of a logarithmic linear model to calculate frequency ratios from a stratified sample.

Pathogen	All patients with pharyngitis (N = 763)			Patients with pharyngitis only (N = 267)		
	Ratio	Percent	Approximate 95 percent confidence intervals* (percent)	Ratio	Percent	Approximate 95 percent confidence intervals* (percent)
C. trachomatis†	23/115	20.5	17 to 26	9/43	20.9	7 to 39
Virus†	21/149	17.7	14 to 23	4/50	8.0	2 to 22
M. pneumoniae†	14/131	10.6	7 to 15	4/45	8.9	2 to 23
Group A streptococcus‡	69/763	9.1	8.5 to 11.5	36/267	13.5	8 to 20
Heterophil-positive	15/763	2.0	1 to 3	9/267	3.4	1 to 7

*Based on size of sample in which the diagnostic test was performed. †Based on data from paired sera. ‡Fourfold increase in antibodies to streptococcus was observed in only 38 percent of patients from whom group A streptococcus was isolated.

as in those patients with only pharyngitis and no symptoms or signs of bronchopulmonary infection (Table 1). In both patient groups evidence of chlamydial and mycoplasmal infection together was found much more frequently than evidence of streptococcal and viral infection together.

Of the patients with positive serologic evidence of chlamydial infection, 83 percent had a fourfold increase in IgG antibodies, 52 percent had a fourfold increase in IgM antibodies, 57 percent had a convalescent IgG titer of $\geq$ 1:128, and 43 percent had an IgM titer of $\geq$ 1:64. None of the patients with evidence of chlamydial infection sought care for symptoms of ocular or genital infection in the months before or after the study visit; also, the distributions of age and sex in these patients did not differ significantly from those of the other patients. Nineteen percent of the patients had evidence of coinfection with viral organisms, 5 percent showed coinfection with group A streptococci, and 1 percent showed coinfection with *M. pneumoniae*. Sixteen percent had evidence of infection with *C. trachomatis* only.

Our data suggest that, contrary to the traditionally held assumption, group A streptococcus may not be the most common potentially treatable cause of pharyngitis in adult patients. Furthermore, penicillin therapy—the preferred antibacterial for streptococcal pharyngitis—is inadequate in the treatment of chlamydial or mycoplasmal infections.

We believe that *C. trachomatis* may be an important pharyngeal pathogen for several reasons. It is a recognized cause of pneumonia in infants (*12*) and may also cause pneumonia in adults (*13, 14*). Furthermore, the organism has been isolated from the pharynx in isolated cases of symptomatic pharyngitis (*15*). In this study we did not attempt to isolate the organism; we used serologic tests of infection only. We cannot exclude the possibility that the serologic evidence of chlamydial infection represented nonspecific polyclonal stimulation, cross-reactivity to antigens from another (undetected) microorganism, or silent chlamydial infection elsewhere in the body. However, we believe it unlikely that such phenomena explain our findings. First, many of the patients had elevated IgM titers—generally regarded as a specific indicator of recent infection. Second, studies of patients with pulmonary infections have not revealed cross-reactivity with antigens of other respiratory pathogens, including adenovirus, respiratory syncytial virus, influenza viruses A and B, parainfluenza viruses 1, 2, and 3, herpesvirus, *M. pneumoniae*, or *Legionella pneumophila* (*16*). Third, serologic evidence of chlamydial infection was not found in any of 33 patients with noninfectious cardiopulmonary diseases (*13*). Fourth, seroconversion or fourfold antibody increases occur in no more than 5 percent of young, culture-negative patients seen in clinics for sexually transmitted diseases, a group at high risk for asymptomatic genitourinary infection with *C. trachomatis* (*17*).

Drainage from the eye through the nasolacrimal duct is one possible route of infection; indeed, five investigators who deliberately inoculated their conjunctivas with *C. trachomatis* all experienced marked pharyngitis within 2 days (*18*), and *C. trachomatis* has been isolated simultaneously from the nasopharynx and conjunctivas of children in areas with endemic trachoma (*19*). Passage from the anogenital area by the hands and through orogenital sexual practices are also possible routes of infection.

Unlike *C. trachomatis, M. pneumoniae* is an established pharyngeal pathogen (*20–22*). However, the frequency of mycoplasma pharyngitis in adults in the clinical setting had not previously been measured. Our study suggests that *M. pneumoniae* causes pharyngitis in adults as often as does group A streptococcus. The frequency of *M. pneumoniae* infection in our study (10.6 percent) was somewhat greater than that generally reported in studies of children, teenagers, college students, and military personnel (*20–22*). However, in one recent study of the cause of acute pharyngitis in college students, *M. pneumoniae* was isolated in 33 percent of the patients (versus only 8 percent of the controls) (*23*). Serologic tests for mycoplasmal infection, such as we used, are regarded as highly specific in patients with respiratory infection (*24*), although false positive reactions sometimes can occur (*25*).

Like other investigators (*20, 22, 26*), we could not identify a pathogenic organism in many patients. Viral infections, in particular, are likely to have been more common than our results suggest. We did not study the value of treatment. Therefore our results should not be taken as justification for the widespread use of antibacterials in patients with pharyngitis or for routinely performing diagnostic tests for chlamydial or mycoplasmal organisms.

A. L. KOMAROFF
M. D. ARONSON
T. M. PASS
C. T. ERVIN
W. T. BRANCH, JR.
Division of General Medicine, Joint Department of Medicine, Brigham and Women's Hospital and *Beth Israel Hospital, Harvard-Thorndike Laboratory, Harvard Medical School, Boston, Massachusetts 02115*
J. SCHACHTER
Department of Laboratory Medicine, University of California Medical Center, San Francisco 94103

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with a throat culture positive for group A streptococci ($N = 63$); (ii) all patients with a pneumonic infiltrate visible on chest x-ray ($N = 8$); (iii) all patients with a sore throat severe enough to interfere with normal daily activities and also with a severe cough ($N = 90$); (iv) all patients with a severe cough (associated with a temperature greater than 101°F, or productive of sputum, or of greater than 14 days' duration, or severe enough to interfere with normal daily activities, or with rales on physical examination) ($N = 186$); (v) a randomly selected 30 percent sample of patients with severe sore throat ($N = 46$); and (vi) a randomly selected 10 percent sample of all patients not meeting any of the above criteria ($N = 64$).

6. There were no significant differences among the groups of patients tested for antibodies to each organism with respect to age, sex, socioeconomic status, symptoms, signs, or resolution of symptoms on follow-up contact ($P > 0.05$ by t-test or χ^2 test, two-tailed).

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Heme-Heme Orientation and Electron Transfer Kinetic Behavior of Multisite Oxidation-Reduction Enzymes

Abstract. *Analysis of the polarized single-crystal absorption spectra of cytochrome cd$_1$ of* Pseudomonas aeruginosa *shows that the heme c and heme d$_1$ groups in each subunit are oriented perpendicularly to each other in both oxidized and reduced forms of the enzyme. These results, together with those of previous kinetic studies, indicate that a perpendicular heme-heme orientation may be an important factor in specifying kinetically slow steps in a sequential series of electron transfer reactions.*

Intramolecular electron transfer is a characteristic feature of the catalytic action of a number of cytochromes containing multiple, prosthetic heme groups (*1–4*). Current theoretical models (*5, 6*) emphasize the importance of heme-heme distance as a controlling factor in such electron transfer processes; however, very little consideration has been given to the role of heme orientation. Spectroscopic (*7–9*) and molecular modeling (*10, 11*) studies suggest that heme groups in multicomponent (and kinetically facile) complexes of cytochromes are aligned in a parallel fashion, and a roughly parallel heme-heme orientation has been found in (diheme) cytochrome c′ through x-ray crystallographic studies (*3*). However, in the tetraheme-containing protein cytochrome c_3, the four heme groups are found in coparallel pairs, each pair being oriented approximately perpendicularly to the other (*4*). We now report, on the basis of polarized single-crystal absorption spectroscopy, that the individual heme c and heme d_1 prosthetic groups in each of the two subunits of nitrite reductase (cytochrome cd$_1$; ferrocytochrome c_{551}: oxygen oxidoreductase, E.C. 1.9.3.3) of *Pseudomonas aeruginosa* are oriented mutually perpendicularly to one another. The implication of this finding, in light of the results of kinetic studies (*12*) of oxidation-reduction reactions of cytochrome cd$_1$, is that heme orientation may be an important factor controlling the rate of electron transfer in multiheme enzymes.

Cytochrome cd$_1$ of *Pseudomonas* is a water-soluble, dimeric protein with two identical subunits of molecular weight 63,000, each with spectroscopically distinct prosthetic groups of heme c (iron protoporphyrin IX) and heme d_1 (iron chlorin) (*13–15*). The enzyme has been crystallized from 70 to 75 percent ammonium sulfate in space group $P2_12_12$ as thin, diamond-shaped plates (*16, 17*). Both heme groups are low-spin ($S = 1/2$) in the oxidized form of the enzyme, whereas the ascorbate-reduced protein contains an ($S = 0$) Fe(II) heme c group and high-spin ($S = 2$) Fe(II) heme d_1 group (*18*). The heme d_1 group is the site of substrate binding and reduction.

The solution absorption spectra of the oxidized and (ascorbate) reduced forms of cytochrome cd$_1$ are illustrated in Fig. 1. Figures 2 and 3 show correspondingly polarized single-crystal absorption spectra. In the crystal spectra the characteristic bands of the heme c chromophore in the visible region are observed primarily in the *b*-crystal spectrum, whereas the absorption bands belonging to the heme d_1 group are observed largely in the orthogonally polarized *a*-crystal spectrum. Thus, except for the strongly overlapping Soret absorption of the heme c and heme d_1 groups, the orthogonally polarized crystal spectra resolve the absorption bands of each of the two types of heme groups in both oxidized and reduced forms of the protein. Comparable observations (data not shown) obtain for crystals of the oxidized enzyme in the presence of 0.2*M* potassium cyanide under which conditions both heme groups are ligated by the cyanide anion (*18*). We have also made similar observations in the case of crystals reduced with sodium dithionite (*19*) or with ascorbate under conditions (0.2*M* potassium cyanide at *p*H 7) in which the cyanide anion is bound only to the heme d_1 group (*18*).

Polarized single-crystal spectroscopic studies of a variety of heme proteins (*20–25*) have established that the heme group exhibits the absorption properties of a square-planar chromophore with respect to the π,π^*–transitions that give rise to the Soret (B) band near 25,000 cm^{-1} and to the Q bands near 17,000 cm^{-1} in both oxidized and reduced states. On this basis, the separate contributions of heme c and heme d_1 to the orthogonally polarized crystal absorption spectra require that the heme c and heme d_1 groups are nearly parallel to the *b* and *a* crystal axes, respectively. Since there is only one pair of spectroscopically distinct heme chromophores in the asymmetric unit of this crystal (*16, 17*), it follows that the heme c and heme d_1 groups are oriented perpendicularly to one another within each subunit of the protein. Coincidence of the molecular dyad with the crystallographic twofold axis specifies further that the heme c group of one subunit is also perpendicular to the heme d_1 group of the neighboring subunit in the dimeric enzyme.