Limitations of Countercurrent Immunelectrophoresis (CIE) in the Diagnosis of Empyema

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Infectious agents are the most common cause of pleural effusions in children, developing more commonly during the course of bacterial pneumonias and less frequently associated with viral, mycobacterial, and fungal respiratory infections. Immunologic techniques, such as countercurrent immunelectrophoresis (CIE), are often more valuable than cultures in establishing a specific etiologic diagnosis in children. In seven of the ten cases of empyema in childhood reported by Siegel et al,1 CIE was positive, while cultures of blood and pleural fluid were negative. Antibiotics had been given in each of these seven cases of sterile pleural effusion. The article by Holsclaw and Schaeffer in this issue of Chest (see page 867) reports the successful use of this technique to identify the specific etiologic agent in sterile pleural effusion in a puzzling case clinically suggestive of rheumatic fever or varicella prodrome. CIE is a rapid and sensitive method for identifying as little as .05 to 0.1 µg of several different types of pneumococcal antigens or Hemophilus influenzae, type B (Hi B) antigen. This is an especially useful diagnostic method when the patient previously has been given antimicrobial drugs or the infection has become subacute.

Countercurrent immunelectrophoresis detects soluble polysaccharide capsular antigens or certain bacteria in pleural fluid and other body fluids by precipitin reactions in gel. Precipitin lines form in the gel media at the point of antigen-antibody interaction under specific conditions of buffer and pH. CIE depends on the antigen with a negative electrical charge and the antibody with a positive electrical charge. When the electric field is applied, the negatively charged antigens migrate toward the anode, and the positively charged ions migrate in the opposite or counter-direction, i.e., toward the cathode.

There are limitations to this valuable diagnostic technique that should be appreciated by those who enthusiastically support its use. False-negative results from CIE might occur when there is a very large quantity of polysaccharide antigen present in the pleural fluid. This is due to antigen excess or prozone phenomena. It is more likely to occur when undiluted empyema fluid is used. The limitation can be alleviated by testing at least two dilutions in saline, e.g., 1:50 and 1:100, in addition to the undiluted pleural fluid.

Another limitation of CIE in the diagnosis of bacterial infections in the pleural fluid occurs when the offending organisms are types 7 or 14 pneumococci. These commonly encountered pneumococcal serotypes are not detected by CIE unless a special buffer is used. The capsular antigens of both types 7 and 14 pneumococci are positively charged, and CIE depends on an antigen with a negative electrical charge and an antibody with a positive charge. These types of pneumococci can be picked up by Ouchterlony precipitin test, which does not depend on the electrical charges of the reactants.

Samples of pleural fluid can be contaminated with excessive amounts of cellular debris; this may result in soft, nonspecific deposits in the gel, which interfere with the detection of specific precipitin bands. Preparation of cell-free supernatant from centrifuged samples of pleural fluid help to circumvent this limitation of the CIE technique. Another potential limitation of CIE is lack of specificity for bacterial capsular antigens because of cross-reactions with other organisms. Reports in the literature, however, indicate that cross-reactions rarely occur.

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when CIE is used in the etiologic diagnosis of empyema.\textsuperscript{2}

The cost of potent and specific antisera has been a factor limiting the use of this valuable diagnostic procedure. Latex particle agglutination may offer advantages over CIE, such as lower cost per test, simpler technique, and greater sensitivity for identification of \textit{H influenzae} type b (Hi B). With the increasing frequency of pleural effusion in children due to \textit{H influenzae} (Hi B), it is important that immunologic techniques, such as CIE or latex particle agglutination, be used to identify infections with this organism so that appropriate antibody therapy can be promptly started.

### Counterimmunoelectrophoresis in the Diagnosis of \textit{Hemophilus influenzae} Pleural Effusion\textsuperscript{*}

\textit{Douglas S. Holsclaw, Jr., M.D.\textsuperscript{t} and David A. Schaeffer, M.D.}

A child with a sterile pleural effusion resulting from an infection with \textit{Hemophilus influenzae} type b (Hi b) is described. The diagnosis was established by use of counterimmunoelectrophoresis (CIE). The alarming increase in incidence of pneumonia due to Hi b is noted, as is the large number of associated pleural effusions. CIE provides a rapid, reliable, and sensitive means by which to establish the exact etiology of such bacterial infections so that optimal antibiotic therapy can be started promptly.

R\textsuperscript{e}cent attention has been called to the increasing frequency in children of pneumonia caused by \textit{Hemophilus influenzae} type b (Hi b).\textsuperscript{1-4} In 1978, Asmar et al\textsuperscript{1} noted that only 77 cases have been reported in the American literature since 1954, to which they added 43 cases diagnosed in the previous 43-month period. Others have reported an additional 112 cases in the past year.\textsuperscript{2-4} In Hawaii, over the past five years, Hi b has become the most common cause of bacterial pneumonia in children.\textsuperscript{5}

Pleural effusion is found frequently in pneumonia caused by Hi b with reported incidences of 9, 31, 75 and 30 percent.\textsuperscript{1-4} However, routine bacterial cultures of pleural fluid obtained by thoracentesis often fail to grow the organism. Lampe et al\textsuperscript{6} have shown that analysis of pleural fluid by counterimmunoelectrophoresis (CIE) can provide a presumptive etiologic diagnosis in more than half of samples with negative bacterial cultures. As the following case report illustrates, the use of CIE may increase the diagnostic yield of pleural fluid specimens by identifying the infecting organism.

### CASE REPORT

A three-year-old boy was admitted to Hahnemann Hospital because of fever of two weeks' duration and a left pleural effusion.

He had been well until two weeks before admission, when he fell and struck the right side of his face. The next day he had a fever and a swollen, ecchymotic right eye. The fever persisted, and examination 11 days before admission revealed exudative pharyngitis, which was treated with cephalaxin. Throat culture was negative for \textbeta-hemolytic streptococci. The fever persisted (39.4\degree C), and the patient's appetite and fluid intake decreased.

After two days of cephalaxin therapy, he was admitted to another hospital because of pain in his left knee and inability to bear weight on his left leg. An x-ray film of the knee was normal, and a technetium bone scan revealed no evidence of osteomyelitis. Chest x-ray film showed lower lobe pneumonia with a left pleural effusion. Thoracentesis was attempted unsuccessfully. Cultures of blood, CSF, and urine were negative. Results of a PPD skin test, gastric aspirate for AFB, heterophile antibodies, and cold agglutinins were negative. His WBC count was 53,000/\textmu mm, with a left shift; ESR was 110 mm/hr. Antistreptolysin O titer was 240 Todd units (normal = less than 100). Antistreptococcal deoxyribonuclease B titer was 2720 units (normal = 0 to 60). Treatment included ampicillin, amoxicillin, and methicillin. Because the chest x-ray findings of pneumonia and pleural effusion persisted, on the 14th day of his illness the patient was transferred to Hahnemann Hospital.

Further history revealed that he had an eight-year-old sibling who had developed chickenpox two days before the onset of the patient's present illness, and a ten-month-old sibling was currently hospitalized with pneumonia of unknown cause. The patient's father had traveled in the

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