Fever of Unknown Origin: Attempts to Isolate L-Forms and Other Aberrant Bacterial Forms

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An investigation was conducted with 65 selected febrile patients, 33 of whom fulfilled in all respects the classic criteria of "fever of unknown origin." Clinical evaluation included extensive radiological and immunological testing. Multiple blood cultures were examined by conventional methods in control studies. In addition, venous blood was cultured in a variety of hyperosmolar media using the special techniques used to detect L-forms and other cell wall-defective bacterial forms. By the extensive methods used, no bacterial forms were isolated. The use of media containing osmotic stabilizers did not detect L-forms or other aberrant bacterial forms, nor did it contribute to the determination of the etiology of fever of unknown origin in these patients.

Several reports in the literature support the application of routine surveillance for the detection of L-forms and aberrant bacterial forms in patients with chronic febrile illnesses of obscure etiology (3, 5). Others refute this search, considering it "unwarranted" (11). Bacterial L-forms are susceptible to antimicrobial agents and are capable of reverting to the parent form (12). The isolation of these organisms from infectious disease processes may have important therapeutic implications. Several publications have emphasized the benefit to the patient who has received therapy directed against aberrant bacterial forms (2, 6, 9). The purpose of this report is to record the results of the attempts to isolate L-forms or other aberrant bacterial forms from the peripheral venous blood of 65 selected patients with pyrexia in whom multiple blood cultures by conventional methods had failed to demonstrate any bacterial growth.

MATERIALS AND METHODS

Blood was cultured from 65 patients with fever of unknown origin, 33 of whom completely fulfilled the classical criteria, as established by Petersdorf and Beeson (10). The patients selected for investigation had been subjected to detailed immunological, microbiological, and radiographic evaluations for persistent, unexplained fever and had not received antimicrobial agents within 1 week of the study.

Body fluids and tissue biopsies were processed so as to demonstrate and/or isolate conventional bacteria, *Mycobacterium tuberculosis*, atypical mycobacteria, and fungi. No viral cultures were performed, nor animal inoculations.

Culture techniques (routine). Venous blood was

inserted into thioglycolate broth containing resazurin and into tryptic digest of casein soy broth (TSB) fortified with 0.03% sodium polyanethol sulfonate resulting in a blood-medium ratio of 1:10. The TSB bottles, containing a vacuum and a CO₂ atmosphere, remained unvented. The blood cultures, incubated at 35°C for 2 weeks, were inspected daily. Blind subcultures were performed from the TSB bottles, 2 and 7 days after the blood had been obtained from the patient. Subcultures, inoculated onto 5% sheep blood agar and 5% horse blood chocolate agar plates, were incubated at 35°C in both a 10% CO₂ incubator and a GasPak anaerobic jar.

Culture techniques. Five to seven milliliters of peripheral venous blood was inoculated simultaneously into 50 ml of brain heart infusion broth containing 10% sucrose and into 50 ml of brucella broth with 30% sucrose. Efforts were made to perform the venipuncture 1 to 2 h before the anticipated rise of the temperature, as well as during the presence of shaking chills. In most cases, the attempt to isolate L-forms and other aberrant bacterial forms was made during the 2nd week of the patient's persistently unexplained febrile state. Patients did not have to experience fever for greater than 3 weeks in duration to be entered into the study.

The blood culture bottles were incubated aerobically at 33°C and examined for bacterial and L-form growth over a period of 30 to 31 days. Bacteriological smears and subcultures to horse blood agar plates were performed on days 4, 10, and 30 or 31, respectively. The medium for the growth of L-forms consisted of Albimi brucella agar (Pfizer) containing 10% sucrose, 10% inactivated horse serum, and 0.5% yeast extract. The concentration of sucrose in the medium varied in some experiments from 10 to 20% (wt/vol) (8). No penicillin or other antibiotics were added to the medium.

Duplicate plates of L-form agar and horse blood

agar were inoculated from the blood culture bottles, on the days specified, with a Pasteur pipette, as these media support the growth of virtually all bacterial L-forms. The duplicate plates were incubated aerobically and anaerobically (Fortner method [10]). All plates were examined at 48 and 96 h with a magnifying lens and microscopically by the Dienes technique for stained agar preparations (1, 7). This method was designed for the study of mycoplasma and bacterial L-forms. The cultures are examined in situ, and L-forms and other cell wall-defective forms are readily visualized and identified by this method.

RESULTS

No L-forms or other aberrant bacterial forms were isolated from the peripheral blood of these 65 patients in spite of the fact that this research laboratory specializes in the study of L-forms and has had considerable experience in their isolation.

Bacterial infection was proven to be the etiology for "fever of unknown origin" in 6 of the 34 patients who fulfilled the classic criteria. Two patients had disseminated tuberculosis, as determined by liver biopsy. One patient had a subphrenic abscess from which *Escherichia coli*, enterococci, and *Bacteroides* sp. were recovered. Another patient had a pelvic abscess, which contained *E. coli* and *Proteus mirabilis*. Two patients had chronic osteomyelitis: vertebral involvement from *Staphylococcus aureus*; prosthetic hip infection due to *Staphylococcus epidermidis*.

DISCUSSION

After an extensive diagnostic evaluation, there remains a modest number of patients who have no obvious disorder to account for their persistently febrile state (4). It has been suggested that these patients represent ideal candidates for the study of the presence of L-forms or cell wall-deficient bacterial organisms and that cultures should routinely be processed to attempt to recover these organisms (5). Our

results do not support this suggestion. We agree with Phair and colleagues that routine cultural surveillance of the peripheral blood for L-forms or other aberrant bacterial forms, as performed by present methods, cannot be justified as an essential element in the diagnostic evaluation of the patient with fever of unknown origin (11).

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