

NOTES

IMMUNOFLUORESCENT STAINING OF *BACILLUS ANTHRACIS* IN DRIED BEEF

JOSEPH Z. BIEGELEISEN, JR.

Communicable Disease Center, Public Health Service, U.S. Department of Health, Education, and Welfare, Atlanta, Georgia

Received for publication 28 February 1964

The demonstration of *Bacillus anthracis* in animal or human tissue has depended largely on the conventional cultural, biochemical, and animal pathogenicity tests. These are often time consuming and complex because of the similarity of the anthrax bacillus to other closely related species of the same genus. In addition, live cells are required to perform these laboratory tests. The Ascoli test (Immunitaetsforsch. **11**:103, 1911) based on the demonstration of a soluble, heat-stable, specific polypeptide antigen has been of value as a test for determining the presence of *B. anthracis* in animal tissues. Despite the intensive research performed on serological methods for the laboratory diagnosis of anthrax infections, no rapid, specific, and sensitive method was reported until 1958 when Levina (J. Microbiol. Epidemiol. Immunobiol. USSR (Engl. Transl. **29**:6, 1958) described the labeling of *B. anthracis* immune globulin and compared the direct fluorescent-antibody (FA) staining technique with the precipitin method. Cherry and Freeman (Zentr. Bakterirol., Parasitenk., Abt. I Orig. **175**:582, 1959) further elucidated procedures for the preparation of reagents for the direct and indirect FA tests for this pathogen in cultures and in human and murine tissue sections and impression smears. Dolgov (Military Med. J. (Engl. Transl.) **10**:57, 1961) also reported on studies of the application of the FA technique to the identification of cultures of anthrax bacilli. Biegeleisen et al. (Am. J. Hyg. **75**:230, 1962) described the rapid identification of the etiological agent of an outbreak of cutaneous anthrax by a combination of animal inoculation and immunofluorescence techniques.

This report deals with the FA demonstration of *B. anthracis* in specimens of dried beef (jerky) used as food. An outbreak of cutaneous anthrax and gastrointestinal disease on an Indian reserva-

tion was reported by P. S. Brachman of the Communicable Disease Center (*personal communication*). Specimens from a freshly butchered steer which had died from unknown causes were submitted to the laboratory. Also, there were samples of stripped, sun-dried beef from the lot consumed by the family involved. The jerky was cut, and the fresh surface was moistened in sterile saline. The processed ends were cultured by touching them to the surface of blood-agar plates, and tissue imprints were prepared on slides. A gram-positive bacillus isolated from the plate culture was identified as *B. anthracis* by conventional tests. The imprints were gently heat-fixed and treated with fluorescein isothiocyanate-labeled *B. anthracis* globulin for 30 min in a moist chamber at room temperature. They were rinsed, mounted, and subsequently examined with a Reichert Fluorex unit fitted with an HBO-200 maximum pressure mercury arc burner and a dark-field condenser. Schott glasses BG-12 and OG-1 were used as primary and barrier filters, respectively. Intensely fluorescent, highly encapsulated bacilli were observed (Fig. 1). The identification of these organisms as anthrax bacilli was confirmed by (i) culturing of the organisms from the same specimens, (ii) the inhibition of their fluorescence in the tissue imprints after treatment with specific antiserum for *B. anthracis*, (iii) the absence of fluorescence inhibition when unlabeled normal serum was substituted for the unlabeled *B. anthracis* antiserum, and (iv) the absence of staining when the imprints were treated with fluorescein-labeled globulin from unimmunized animals.

The procedure described here is a simple method of screening tissue specimens rapidly for demonstrating the presence of the vegetative phase of *B. anthracis*. This method should find application in laboratories concerned with the

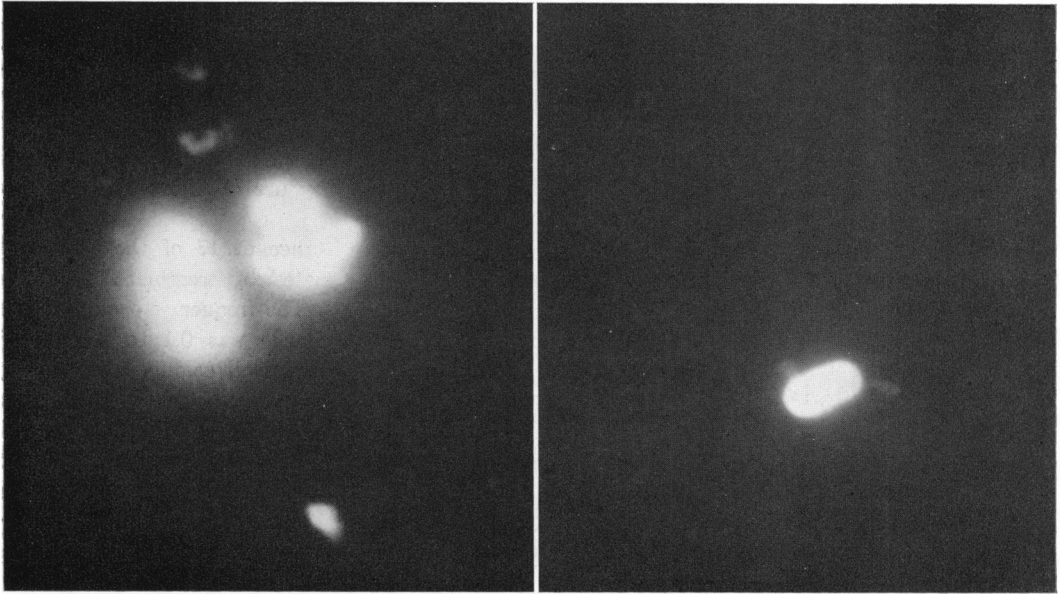


FIG. 1. Fluorescent-antibody staining of *Bacillus anthracis* in a tissue imprint of dried meat. Due to the intense staining of the large quantity of capsular material, the organisms have a hazy appearance. 1,250 X.

diagnosis of anthrax, since the organisms can be detected with rapidity in the tissues and lesions. Therapeutic and control measures then can be instituted. The presence of viable organisms is not a requirement for application of the

test. Attention should be given to the epidemiological and clinical features of the disease, since the fluorescence of some nonpathogenic, gram-positive, sporeforming bacilli superficially may resemble that of *B. anthracis*.

ELIMINATION OF PENICILLIN RESISTANCE OF *STAPHYLOCOCCUS AUREUS* BY TREATMENT WITH ACRIFLAVINE

HAJIME HASHIMOTO, KEI KONO, AND SUSUMU MITSUHASHI

Department of Microbiology, School of Medicine, Gunma University, Maebashi, Japan

Received for publication 17 February 1964

In a previous paper (Mitsuhashi et al., *J. Bacteriol.* **86**:162, 1963), it was reported that the macrolide (erythromycin, oleandomycin, leucomycin) resistance of *Staphylococcus aureus* was eliminated by treatment with acriflavine and that its resistance was transduced with phage. These facts strongly suggest that macrolide resistance of *S. aureus* is controlled by one genetic element of extrachromosomal nature.

Voureka (*J. Gen. Microbiol.* **6**:352, 1952) reported that the resistance to penicillin and streptomycin of a strain of *S. aureus* decreased after

exposure to injurious agents, such as chloramphenicol, tetracycline, and nitrogen mustard. It was also reported that the capacity of *S. aureus* to produce penicillinase was transduced by typing phage (Ritz and Baldwin, *Proc. Soc. Exptl. Biol. Med.* **107**:678, 1961).

Novick (1963) obtained evidence to suggest that the penicillinase inheritance of *S. aureus* is extrachromosomal. This paper deals with the elimination of the penicillin resistance of *S. aureus* by treatment with acriflavine.

Eighteen strains of *S. aureus* were used. They