TABLE 1. *Number of recombinants formed in crosses between lysogenic (P_1) and nonlysogenic strains and recovery of xyl^+ and tpa^+ in the mal^+ recombinants**

Cross	Male × female	No. of mal^+ recombinants	No. of ilv^+ recombinants	Per cent mal^+ recombinants that are	
				xyl^+	tpa^+
I	AB2229 × AB1450	6×10^6	1×10^5	90	Not done
II	AB2229 × AB2147 (P_1)	1×10^6	3×10^2	10	8
III	AB2154(P_1) × AB2147(P_1)	3×10^6	5×10^4	82	68
IV	AB2154(P_1) × AB1450	7×10^6	1×10^5	80	Not done

* Abbreviations: *mal*, maltose; *lac*, lactose; *xyl*, xylose; *ilv*, isoleucine and valine; *tpa*, tryptophanase; (P_1) lysogenic for bacteriophage P_1 .

broken down into smaller pieces in the zygotes before integration has occurred. Thus, the physical linkage between mal^+ and the two proximal markers, xyl^+ and tpa^+, is destroyed in the restrictive cross. Boyer (Bacteriol. Proc., p. 31, 1964) also observed a reduction in linkage, as a result of restriction, when *E. coli* B/r is crossed with *E. coli* K-12.

IMMUNOFLUORESCENCE TECHNIQUES IN RETROSPECTIVE DIAGNOSIS OF HUMAN LISTERIOSIS

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Received for publication 27 January 1964

Listeria monocytogenes was first reported to be the etiological agent of infections in animals (Murray, Webb, and Swann, J. Pathol. Bacteriol. **29**:407, 1926). The first recognized case of human disease resulting from infection with this organism was published 3 years later (Hyfeldt, Compt. Rend. **101**:590, 1929). Listeric infection resulting in stillbirth or abortion is not common in this country and, since organs and tissues obtained from fetal or neonatal death are not routinely examined bacteriologically, specimens are not always submitted to the clinical laboratory. However, the diagnosis of listeriosis cannot be made without a definitive identification of the organism. Although the physical and physiological characteristics of *Listeria* are clearly defined, the organism may be confused with other related species, especially when observed in histological preparations where *L. monocytogenes* may occur singly, in groups of short chains, or as coccoidal rods in palisade arrangement. Bacteriological identification depends upon recovery of the pathogen from the clinical specimen, a procedure which may be extremely difficult. The organism is serologically type-specific, but its identification requires isolation in pure culture and preparation of suitable suspensions for performance of agglutination tests. Therefore, a rapid, sensitive, highly specific test which does not require viable organisms would facilitate the laboratory identification of *L. monocytogenes* in tissue imprints or sections prepared at necropsy.

The globulin fractions of *L. monocytogenes* antiserums prepared against serotypes I, II, III, IV A, and IV B were labeled with fluorescein isothiocyanate (Riggs et al., Am. J. Pathol. **34**:1087, 1958) according to a modification of the method of Coons and Kaplan (J. Exptl. Med. **91**:1, 1950). The reagents (conjugates) were tested for specificity by (i) staining heterologous species of gram-negative and gram-positive bacteria, (ii) staining heterologous serotypes of *L. monocytogenes* with type-specific conjugates, (iii) blocking the fluorescence reaction by the use of the one-step inhibition test (Goldman, J. Exptl. Med. **105**:557, 1957), and (iv) treating the organisms with conjugated normal rabbit globu-

lin. No cross-staining reactions were observed among the heterologous species, and the *Listeria* cells did not fluoresce when treated with the normal globulin conjugate. It was observed that fluorescent reagents for each of the serotypes designated I, II, and III stained both homologous and heterologous serotype cultures with equal intensity at the working dilution. Despite the fact that serotypes IV A and IV B share a common antigen, strains of these types were stained only with the respective, specific conjugate.

The use of the fluorescent-antibody technique has been reported as a method of identifying *Listeria* in culture (Smith, Marshall, and Eveland, Proc. Soc. Exptl. Biol. Med. **103**:842, 1960; Biegeleisen, Proc. 2nd Symp. Listeric Infection, p. 183, 1963), in animal tissues (Smith and Metzger, Proc. 2nd Symp. Listeric Infection, p. 179, 1963), and in cerebrospinal fluid specimens (Eveland, J. Bacteriol. **85**:1448, 1963). Although the use of labeled antibody was suggested as a means of confirming the clinical diagnosis of listeriosis by staining the organism in paraffin-embedded tissues (Potter, *Pathology of the Fetus and Infants*, 2nd ed., Year Book of Medical Publishers, Inc., Chicago, 1961), the first published report of the use of the technique for this purpose appeared in 1963 (Villella, Halling, and Biegeleisen, Am. J. Clin. Pathol. **40**:151, 1963). In the latter report, there is described a clinically typical case of listeriosis of the newborn in which retrospective confirmation of the clinical diagnosis was made by use of immunofluorescent techniques. Type IV A conjugate specifically stained the organisms in the deparaffinized sections. This case was reported from Hawaii. Subsequently, tissue sections from two additional cases originating in the continental United States have been examined similarly. In both instances, the organisms in the prepared slides stained brilliantly with the polyvalent reagent (consisting of labeled antibodies to serotypes I, IV A, and IV B) and with serotype I conjugate. Since strong cross-staining reactions occur among serotypes I, II, and III, no specific type determination could be made. Types I and IV B are predominant in this country; thus, the indications are that the infections were probably due to *L. monocytogenes* of type I.

The evidence suggests that immunofluorescent methods can contribute important information in clinical pathology laboratories, especially when specimens are not available for conventional bacteriological tests and diagnosis must be made in retrospect. The method has great potential for use in screening specimens obtained from the genital tract of pregnant women who may be colonized or infected with *L. monocytogenes*. Such information may aid in preventing fetal loss resulting from transplacental infection by this organism. It is emphasized that a firm laboratory diagnosis of listeric infection depends upon conventional methods of isolation and identification of the causative organism. Immunofluorescence techniques should be applied for rapid screening of specimens to yield a presumptive diagnosis, or used when bacteriological or other serological procedures cannot be performed.

MICROCOCCUS CRYOPHILUS IS NOT FLAGELLATED

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Received for publication 31 January 1964

McLean, Sulzbacher, and Mudd (J. Bacteriol. **62**:724, 1951) stated in regard to *Micrococcus cryophilus*, "Peritrichous flagella have been demonstrated on a very occasional cell by Kulp's modification of Loeffler's stain. Likewise, an occasional motile cell has been observed in hanging drop preparations."

The first author was solely responsible for the conclusion that this organism was flagellated. The statement quoted above was based on an original stain, even though numerous subsequent stained preparations (Kulp's and Leifson's methods) and electron micrographs made prior to publication failed to demonstrate flagella. After