partly covered and allowed to dry for 30 min, then covered completely and incubated. The plates are read after 24 hr of incubation. Reading is accomplished by removing the cover and placing the plate face up on an illuminated colony counter (Darkfield Quebec colony counter, American Optical Co., Buffalo, N.Y.). The location of the phages is determined by matching the code stamped on the plastic dish with the code employed for stocking the reservoir tray.

The sequence of phage application followed by application of the test strain can be repeated as often as necessary. The time required for application of all of the phages used is approximately 10 to 15 sec per plate. In 2 hr, 100 plates can be completely and conveniently processed. Several hundred strains can be phage typed in 1 day. The volume could be increased if more than one technician were employed. The application of phages and of bacterial suspensions can be staggered.

The reservoir tray is cleaned by immersion in 95% ethanol for 30 min, flushing with tap water, drying, reimmersion in 95% ethanol, and exposure to ultraviolet light for 45 min. The press plate is cleaned by immersion in 95% ethanol for 30 min, washing with tap water, and exposure to ultraviolet light for 45 min. When not in use, the apparatus is kept on an open shelf. Immediately before use, it is rubbed with 80% ethanol and exposed to ultraviolet light for 45 min, while the remainder of the requisite equipment and materials is accumulated.

Contamination is considerably less with this method than with multiple-syringe techniques. Cross contamination of phages on the typing plates or in the reservoir tray has not caused problems. The load on the dish-washing facility, syringe breakage, and the cost of disposable syringes have been eliminated entirely.

The reproducibility of our own results, coupled with comparison test results between our laboratory and others (courtesy of John E. Blair, Hospital for Joint Diseases, New York, N.Y.), have shown this method to be reliable. The original apparatus is still in use after 3 years and shows no signs of wear.

The complete apparatus can be purchased from Sierra Scientific Associates, Albany, Calif., under the name of Identi-Phage. The cost is approximately $50.

ADDENDUM

After submission of the manuscript, we were made aware of the fact that a replicate-plate mechanism similar to the one we describe has been used previously for antibiotic-sensitivity testing (Steers, Foltz, and Graves, Antibiot. Chemotherapy **9**:307, 1959). The limitations of this apparatus for bacteriophage typing have been discussed (Zierdt, Fox, and Norris, Am. J. Clin. Pathol. **33**:233, 1960), and include contamination incurred during the process of air-drying, variation in drop size, phage scatter when the plate is flooded with bacterial suspension, and droplet spatter causing mixing of phages in the reservoir cups. As indicated, we have had essentially no such problems during the more than 3 years of continuous use. We have not seen any published reports concerning the application of these techniques to bacteriophage typing, although both of the above papers indicate that such application has been made. Both sources quote personal communications from S. Goldberg.

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DEMONSTRATION OF *LISTERIA MONOCYTOGENES* IN DIRECT EXAMINATION OF SPINAL FLUID BY FLUORESCENT-ANTIBODY TECHNIQUE

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Many reports have been made on *Listeria monocytogenes* as the causative agent of cases of meningitis (Seeliger, *Listeriosis*, p. 132, Haffner Publishing Co., New York, 1961) in both infants

and adults. Since the meningitis caused by this organism cannot be differentiated from other types of bacterial inflammation except by cultural techniques, delays in recognition of the bacillus as *L. monocytogenes* may often hinder prompt treatment of the cases.

With the development in recent years of the use of fluorescent-antibody techniques for the prompt recognition of various pathogens (Kendrick et al., Am. J. Diseases Children **101**:149, 1961; Page et al., Am. J. Diseases Children **101**:155, 1961; Wolfe and Cameron, Public Health Lab. **17**:76, 1959; Whitaker et al., Pediatrics **27**:214, 1961), it was decided to attempt the use of a polyvalent type of antiserum to identify *L. monocytogenes* in suspected clinical specimens.

Polyvalent antiserum was prepared by inoculation of rabbits with boiled pooled cultures representing types 1, 2, 3, 3b, 4a, and 4b. The antiserum was processed by precipitation of the globulins with saturated ammonium sulfate, and conjugation with fluorescein isothiocyanate, using 0.025 mg of dye per mg of protein, and finally passed through a diethylaminoethyl cellulose column according to the method of Riggs et al. (Proc. Soc. Exptl. Biol. Med. **105**:655, 1960).

Smears prepared from the sediment of a centrifuged specimen of spinal fluid from a case of meningitis were heat-fixed and stained for 30 min at room temperature with the above conjugate to which had been added a Lissamine rhodamine counterstain (Smith et al., Proc. Soc. Exptl. Biol. Med. **102**:179, 1959). Examination of the smears was made with a Zeiss fluorescent microscope.

One to numerous organisms were observed in

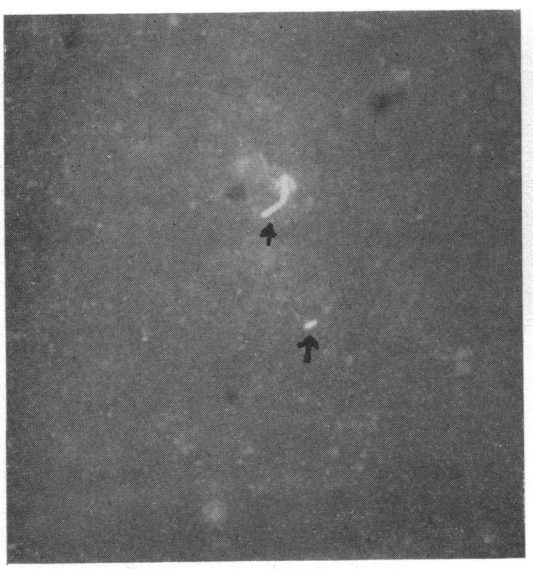

FIG. 1. *Three Listeria monocytogenes organisms among cell debris of smear.*

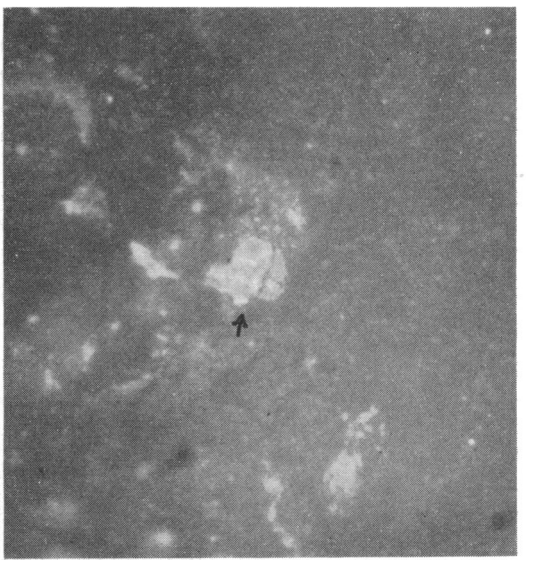

FIG. 2. *Cell containing numerous intracellular Listeria monocytogenes organisms. Note one extracellular form close by.*

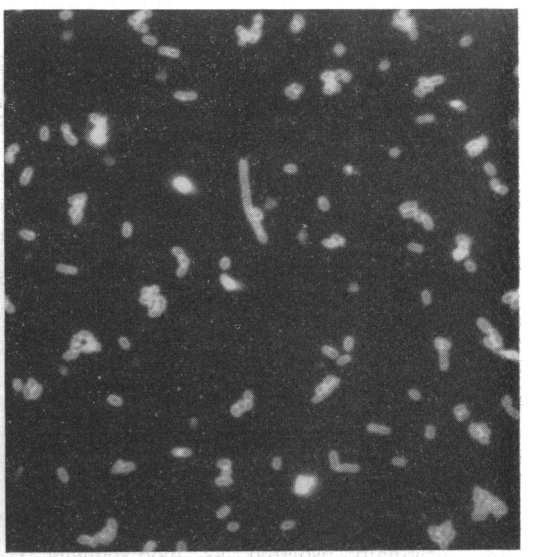

FIG. 3. *Pure culture of Listeria monocytogenes. Note pleomorphism of the organism.*

almost every field examined (Figs. 1 and 2). The stained organisms stood out very clearly against the counterstained background on the smear. It was apparent that the organisms were pleomorphic in this preparation, since both rather long rods and coccobacillary forms were observed.

Confirmation of the presence of *L. monocytogenes* in this specimen was made by culture. The isolated strain satisfied the criteria for *L. monocytogenes* and was designated type 1 by M. L. Gray, Montana State College, Bozeman. It stained very readily with this conjugated polyvalent antiserum (Fig. 3).

I wish to thank Norma Broom, Herman Kiefer Hospital, Detroit, Mich., for help in obtaining this specimen.

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SULFATE-FREE GROWTH OF *CLOSTRIDIUM NIGRIFICANS*

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Certain strains of the mesophilic, nonsporulating sulfate-reducing bacteria (*Desulfovibrio desulfuricans*) grow without detectable sulfate if pyruvate is the main substrate [Postgate, Research (London) **5**:189, 1952] but the sporulating mesophile *D. orientis* does not (Adams and Postgate, J. Gen. Microbiol. **20**:252, 1959). This note reports evidence that the sporulating thermophilic sulfate-reducing bacterium *Clostridium nigrificans* (Campbell, Frank, and Hall, J. Bacteriol. **73**:516, 1957) is capable of sulfate-free growth with pyruvate.

Strains were tested in the basal medium of Postgate (1952), without the 2.5% NaCl there prescribed and with 54 mM sodium lactate or pyruvate (reagent grade) and 27 mM Na_2SO_4. Nine strains were examined: Teddington Garden (NCIB 8351), Delft 74T [NCIB 8395; incorrectly named "14T" by Campbell et al. (1957)], both originally isolated as "*Sporovibrio thermodesulfuricans*"; ATCC strain 3750, ATCC strain 7946, both originally isolated as food-spoilage *C. nigrificans*; strains 55, 106, 134, "By," and "Dp" from the personal collection of L. L. Campbell. All strains were pure according to the criteria of Postgate (J. Gen. Microbiol. **9**:440, 1953). The strains were maintained under N_2 at 55 C in Baars's lactate-sulfate medium (Postgate, J. Sci. Food Agr. **10**:669, 1959) containing 0.1% yeast extract; supernatant fluids from 24- to 48-hr cultures were used as inocula for growth tests. Growth was assessed turbidimetrically in a Klett-Summerson photoelectric colorimeter at 660 mμ.

All nine strains grew in pyruvate media with or without sulfate in less than 18 hr; on prolonged incubation, some decline in optical density occurred with most strains. Growth with lactate was sometimes delayed up to 24 hr and never appeared earlier than with pyruvate; growth did not occur with lactate but no sulfate. Examples of growth yields are recorded in Table 1; strain 55 regularly gave low cell yields with lactate but gave "normal" yields with pyruvate. Data demonstrating the dependence of growth of strain Teddington Garden on pyruvate concentration in the sulfate-free medium are also included in Table 1; this experiment was performed employing the specially purified sodium pyruvate mentioned below. Six strains (those listed in Table 1 with "By" in place of 55) were subcultured six times in sulfate-free pyruvate medium without change in the cell yield; on the sixth passage, they were also returned to Baars's medium, in which they grew and reduced sulfate readily.

For the fourth subculture, the sodium pyruvate was recrystallized from 80% ethanol and the cultures were analyzed after growth. Acetate, the sole volatile acid produced, was characterized by paper chromatography in *n*-butanol + 1 N

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