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# Unsuccessful Attempt to Detect *Listeria monocytogenes* in Healthy Pregnant Women

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*Listeria monocytogenes* has been shown to produce abortions and premature births in experimentally infected animals (M. L. Gray, C. Singh, and F. Thorp, Proc. Soc. Exptl. Biol. Med. **89**: 163, 1955) and has been implicated frequently as a cause of similar complications in human pregnancies (F. Rappaport, M. Rabinovitz, R. Toaff, and N. Krochik, Lancet **7137**:1273, 1960; M. L. Gray, H. P. R. Seeliger, and J. Potel, Clin. Pediat. **2**:614, 1963). Relatively limited data are available concerning the occurrence of the organism in healthy gravid females (M. Hood, Symp. Listeric Infection, 2nd, p. 279, 1962; H. P. R. Seeliger, *Listeriosis*, Hafner Publishing Co., New York, 1961). In this paper are reported the results of a survey of 116 pregnant women randomly chosen from those receiving examinations in the obstetrics-gynecology clinic of Grady Memorial Hospital, Atlanta, Ga., during the period May–August 1965. Both cultural and fluorescent-antibody (FA) procedures were used for direct screening of cervical-vaginal smears, and the latter test was employed as an aid in the identification of organisms cultured from the swabs.

A sterile cotton swab was used to take cervical-vaginal specimens which were plated on modified McBride's medium (phenyl-ethyl alcohol base + 1% glycine + 0.05% LiCl), and the same swab was used to prepare direct smears for FA examination. The swab was then held at 4 C in an atmosphere of 5 to 10% $CO_2$, in 5 ml of tryptose broth, for subsequent plating. Two smears were examined immediately and two were stored at −20 C and examined after 5 to 6 months. A pool of globulin conjugates known to be active against types 1, 2, 3, 4a, and 4b *L. monocytogenes* was used to stain all smears. Swabs were plated after 2 weeks and 1, 2, 3, 4, and 6 months of cold enrichment, following a suggestion that cold enrichment for shorter periods of time had proved inadequate in previous studies (H. P. R. Seeliger, Symp. Listeric Infection, 2nd, p. 318, 1962). Plates were incubated at 37 C for 3 days and then were held at room temperature for an additional 7 days before being discarded. Daily examinations were made by use of Gray's oblique lighting technique. Colonies resembling *Listeria* were screened for motility in semisolid agar (P. R. Edwards and D. W. Bruner, Kentucky Agr. Expt. Sta. Circ. 54, 1942). These were discarded if they failed to produce the typical umbrella or skirtlike pattern of motility associated with a microaerophilic band of growth beneath the surface of the agar. Gram stains, FA stains, and appropriate biochemical tests also were used as an aid in identification.

All specimens were negative for *L. monocytogenes* by cultural methods. The cultural techniques used here are believed to be adequate, since they were identical to those employed a few months earlier for isolation of *L. monocytogenes* from 10 of 12 animal brains which had been frozen for 1 year prior to their examination (B. Pittman and W. B. Cherry, *unpublished data*). In that study, all positive specimens had yielded the organism by the 5th month of cold enrichment and were still positive at the end of 1 year.

Direct FA examination of the smears showed brilliantly stained (4+) short rods or cocci in two specimens. In one of these, the organism causing fluorescence was isolated and identified as *Streptococcus faecalis*. After repeated subculture, the *Streptococcus* showed a marked decrease in fluorescence with *Listeria* conjugate. The stained organisms seen in the second specimen resembled those seen in the first, but were not isolated. Some FA cross-reactions were observed between the *Listeria* conjugate and *Staphylococcus* species and yeasts.

The hospital records of 102 of the patients were checked to correlate the results with past histories of abortions or premature births and with the stage of pregnancy at time of examination. Of the 69 patients in this group who were not primigravida, 15 previous premature births, 17 previous spontaneous abortions, and 199 previous full-

term deliveries were reported in a total of 237 previous pregnancies. Information could not be obtained for six pregnancies. Nine of the patients were in the first trimester of pregnancy, 72 were in the second, and 17 were in the third trimester. The stage of pregnancy could not be determined for 4 patients.

In summary, 116 gravid females were examined for *L. monocytogenes*. All were found to be negative for the organism by both cultural and FA techniques. A strong FA cross-reaction with *S. faecalis* was observed with a pool of FA conjugates for *L. monocytogenes*. Past histories of these patients showed that this population had a sufficient number of previous premature births and spontaneous abortions to suspect possible *Listeria* infections. Most of the specimens were obtained during the last 5 months of pregnancy, the time during which infection is most likely to become evident. In addition, four bacteriologically proven cases of neonatal listeriosis occurred at the hospital during 1964 and 1965 (A. Nahmias, *personal communication*). None of the mothers of the above patients was examined in the present study. Therefore, we conclude that this population, at the time of examination, was not carrying *L. monocytogenes* in the reproductive tracts.

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