SURVIVAL OF *LISTERIA MONOCYTOGENES* IN SOIL[1]

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The hypothesis of soil serving as a natural reservoir for *Listeria monocytogenes* is not unattractive, since the distribution of the organism over an area ranging from the Arctic to Africa involving man and many other species of animals suggests a common source of infection (Murray, 1955; Seeliger, 1955; in a *personal communication*, Dr. M. L. Gray, who has kept an up-to-date record of Listeriae isolations, lists 29 animals, 14 birds, and 1 trout). The title of a paper by Olsuf'ev and Petrov (1949) suggests the isolation of Listeriae from spring water. Gray (1960) reports the isolation of Listeriae from mice that were fed silage which previously was implicated in listeric abortion of cattle. Although the organism has not been found in the soil, some have considered it possible (Seeliger and Cherry, 1957). The present paper is concerned with the survival of *L. monocytogenes* in soil under experimental conditions.

MATERIALS AND METHODS

L. monocytogenes of the serotype 1 was used in these experiments. Cells were grown on tryptose blood agar base (Difco) containing 1 per cent glucose. Inocula for soils and other materials were prepared by growing the Listeriae on the above medium for 24 hr at 34 C, after which time the cells were harvested and washed three times in distilled water by centrifugation. The organisms were allowed to remain in the distilled water only as long as necessary to perform the washing and complete the inoculations.

To represent extremes, two types of soil were selected: clay soil and fertile garden soil. A batch of each type was collected and stored in sealed vessels; therefore all samples were portions of the same specimens throughout the studies.

Soil (3 g) was placed in test tubes, 20 by 120 mm. In some experiments the tubes were capped with Morton culture tube closures and sealed with several layers of Parafilm, whereas in other experiments they were plugged with cotton.

The soils in all cases were inoculated with a suspension containing approximately 10^8 Listeriae in a volume of 0.1 ml. The inoculated soils were held at 24 C to 26 C throughout the experiments.

The numbers of surviving organisms were determined at the designated intervals after suspending each of the 3-g samples in 10 ml of sterile distilled water. After the settling of the large particles, successive 10-fold dilutions of the supernatant suspension were made and the diluted material was cultured in duplicate on tryptose-glucose agar plates by spreading 0.1 ml over the surface with a wire spreader. After incubating for 48 hr at 34 C, those plates having between 30 and 300 colonies were counted. The number of organisms recorded in each experiment represent the Listeriae per ml of the suspended soil sample. Two tubes of each type of soil were sampled at each interval.

RESULTS

In view of the possible modifying effects of soil flora it was considered desirable to inoculate untreated (i. e., unsterilized) soil with Listeriae; however, it quickly became apparent that the massive overgrowth of the soil organisms precluded the detection of Listeriae, even by such refinements as examination by the oblique lighting technique (Gray, 1957); therefore only autoclaved soil was employed.

Duplicate series of tubes, containing fertile soil and clay soil, respectively, were inoculated with Listeriae, plugged with cotton, and tested for survivors at intervals for 67 days. As recorded in figures 1 and 2 the decline of survivors in the clay soil was initiated sooner than in the fertile soil; however, the trend is downward in both instances with some samples of each having <10 to 0 bacteria per ml at the end of 67 days. Since the soils were placed in tubes plugged with cotton

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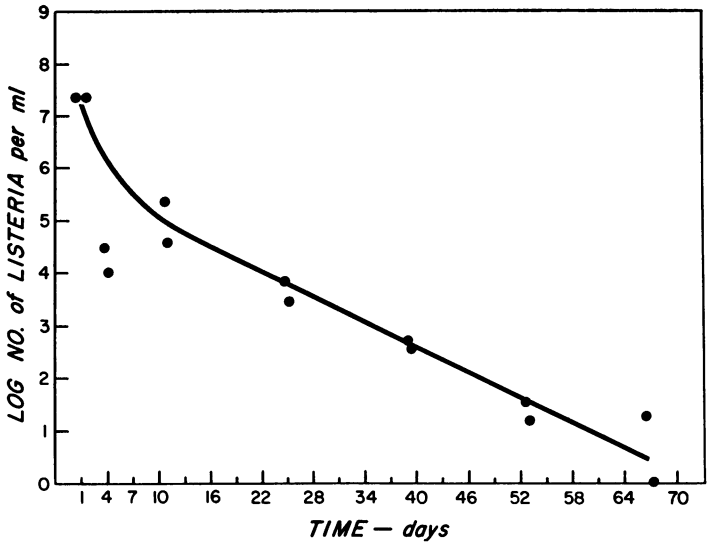

Figure 1. Number of surviving cells of *Listeria monocytogenes* in clay soil held at 24 to 26 C in tubes plugged with cotton.

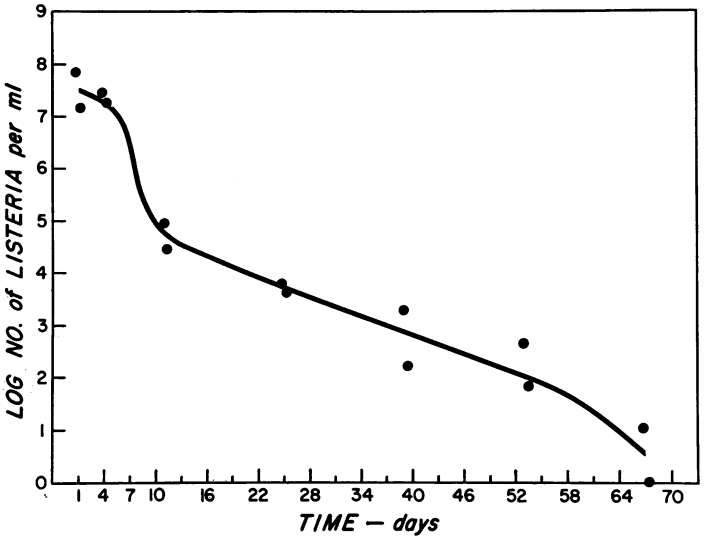

Figure 2. Number of surviving cells of *Listeria monocytogenes* in fertile soil held at 24 to 26 C in tubes plugged with cotton.

it is conceivable that dehydration might have contributed in some measure to the destruction of the organisms. It was determined that the fertile soil and clay soil possessed moisture contents of 17 per cent and 7 per cent, respectively, before inoculation. Samples of the same soils were held under similar environmental conditions in cotton plugged tubes for 165 days and the moisture content was determined. The fertile soil lost 82 per cent of its moisture and the clay lost 75 per cent of its moisture. Samples of soils maintained under the same conditions, but in the capped and sealed tubes, retained all moisture over the same period; consequently all subsequent tests were performed on soils held in the sealed tubes.

Another series of clay and fertile soils were inoculated with Listeriae and held for 44 weeks

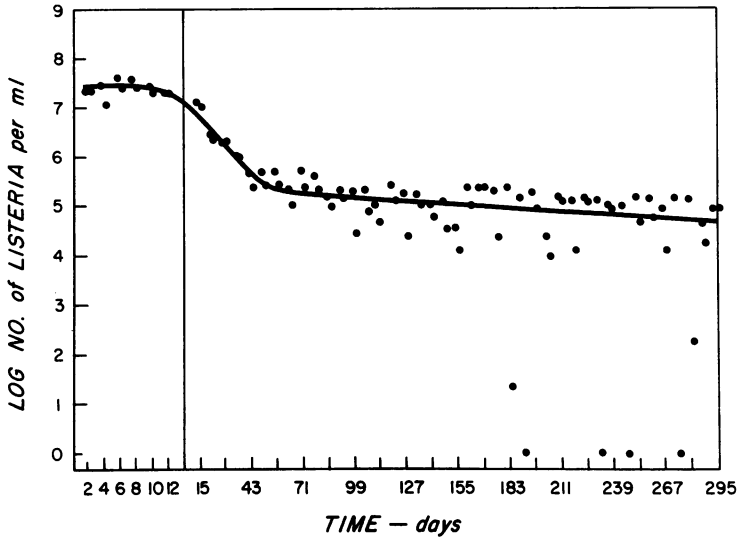

Figure 3. Number of surviving cells of *Listeria monocytogenes* in fertile soil held at 24 to 26 C in sealed tubes.

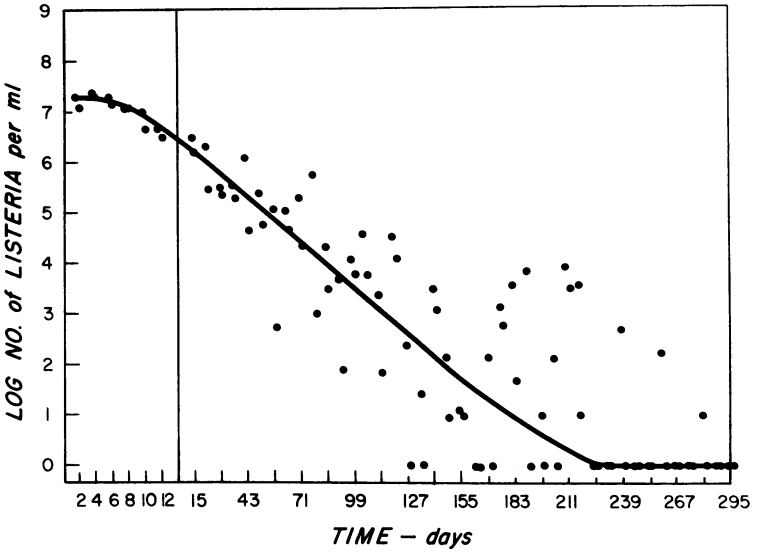

Figure 4. Number of surviving cells of *Listeria monocytogenes* in clay soil held at 24 to 26 C in sealed tubes.

in tubes that were capped and sealed. Two tubes of each type of soil were sampled at intervals over this period and the numbers of surviving Listeriae were determined. The results depicted in figures 3 and 4 show marked differences in the ability of Listeriae to survive in these different soils. The number of viable organisms in the fertile soil remained relatively stationary at approximately 10^7 per ml for the first 15 days after seeding. There occurred a decline to 10^5 organisms per ml in the course of the next 2 months, after which the number of viable organisms remained at this level for the duration of the experiment. One of the samples, at day 183, had <10 to 0 organisms per ml as did three other samples on subsequent days; however the other samples taken on the same days contained approximately 10^5 bacteria per

ml. The number of surviving organisms on the clay soil steadily declined and approached the zero level approximately 6 months after inoculation. During the last 100 days of the experiment <10 to 0 bacteria per ml remained in 25 of the 32 tubes samples. The 7 tubes in which >10 survivors remained had 10^4 to 10^1 Listeriae per ml with the fewest organisms present in the latest samples.

Infusions of the fertile soil were prepared to determine if the sterile soil could serve as a source of nutrients or as a source of inhibitors. These were prepared according to the directions of James (1958) who employed soil infusions for the cultivation of soil organisms. Agar containing soil infusion as nutrient was inoculated with Listeriae. No growth occurred after incubation at 34 C for 48 hr followed by holding at 24 to 26 C for 12 days. No inhibition of growth was detected when tryptose-glucose agar was prepared with the soil infusion comprising the fluid constituent of the medium. On the basis of these limited observations we see no indication that the sterile fertile soil supports or suppresses the development of Listeriae.

To observe the survival of Listeriae on an "inert" substance under similar conditions, glass beads, approximately 250 μ in diameter were employed in place of the soil. The beads were inoculated and held in sealed tubes for a period of 68 days during which time viable cell counts were performed at intervals. The number of viable Listeriae dropped from the initial number of 10^7 per ml to 10^2 per ml by the 20th day of holding. Between the 20th day and 44th day, the number declined to 40 to 0 bacteria per ml. No organisms were recovered from the 44th to the 68th day in any of the samples.

DISCUSSION

The observations of others point to the ability of *L. monocytogenes* to survive under conditions that ordinarily are considered less than optimal for most vegetative forms of bacteria. Gray, Stafseth, and Thorp, (1951) found that cultures maintained on blood agar slants for 3 years without transfer were lethal when injected into rabbits. They also recovered viable Listeriae from medulla suspensions kept in the laboratory at room temperature for 4 years. (Dr. Gray (*personal communication*) states that the same suspensions continued to harbor large numbers of viable Listeriae when tested 10 years later.) These same workers inoculated such materials as rabbit food pellets, hay, straw, and wood shavings with Listeria, and they recorded survival times of 172, 112, 42, and 42 days, respectively. If these figures are compared with the results of the present study, one notes that the survival times are comparable for straw, wood shavings, and glass beads. Clay and fertile soils, unprotected from evaporation of moisture, support viable cells for about a month longer than the straw and shavings. Clay soil in sealed containers is comparable to the rabbit food pellets in that survivors were few after 6 or 7 months. On the other hand, fertile soil protected from evaporation maintained an abundance of survivors for the duration of the observations. The number of organisms at the termination of the experiment after 295 days was only slightly less than at the 43rd day after inoculation.

There was no indication of colony dissociation of the survivors on either clay or fertile soil. Under the conditions of the experiments, sterile fertile soil acts as a maintenance menstruum in which the multiplication and destruction of Listeriae is minimal in contrast to clay soil and glass beads on which the population steadily declines to an insignificant number.

In addition to the foregoing findings, the ability of *L. monocytogenes* to grow at low temperatures (reaching its peak of log phase of growth in 10 to 11 days at 6 C) and its tolerance of high temperatures (withstanding pasteurization by the holding technique) supports the contention that the organism is endowed with properties that should favor its survival in soil and other areas outside of the animal host (Bearns and Girard, 1958).

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SUMMARY

The number of surviving cells of *Listeria monocytogenes* was determined periodically after inoculation into sterile fertile soil and sterile clay soil held at 24 to 26 C. The survival of *L. monocytogenes* in these soils was observed to be dependent upon the type of soil involved and the moisture content. Where loss of moisture was not controlled, the number of Listeriae in

both soils declined steadily and at approximately the same rate until few if any organisms survived after 67 days. In fertile soil protected from evaporation, there was a decline of Listeriae during the first month; thereafter the number remained almost stationary at a relatively high level for the remainder of the 295 day period of observation. The number of Listeriae in clay soil, in which drying was avoided, steadily declined until most samples failed to show survivors after 200 days.

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