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Listeria monocytogenes and Listeric Infections

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INTRODUCTION

Listeriosis is one of the most recently recognized and least understood of all the bacterial infections of man, his domesticated animals, and the wildlife which share his cities, farms, and recreation areas. Although more than three decades have passed since Murray et al. (164) first described in detail the bacterium known

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today as *Listeria monocytogenes*, the passing of 36 years has added comparatively little to our knowledge of either the bacterium or the host of disorders with which it has been associated. In spite of a bibliography that now numbers well over 1,600 items, it is thought by many people to be of little or no economic importance. It is often relegated to the class of laboratory curiosities—a bacterium that can produce a high circulating monocytosis of the peripheral blood or a marked purulent conjunctivitis in a susceptible laboratory animal. But those who have studied it closely see it as a potential menace, an indiscriminate killer of young and old alike, and, until the advent of sulfa drugs and antibiotics, individuals known to survive its attack were usually left with permanent physical or mental defects.

The astonishingly wide host range includes at least 37 mammals in addition to man, 17 fowls, ticks, a fly caught in a laboratory, fish, and crustaceans. It has been found in stream water, mud, sewage, slaughter house waste, silage, and sickroom dust, and has world-wide distribution. Even though *L. monocytogenes* is apparently distributed widely, the sporadic occurrence of the various forms of listeric infection suggests that its distribution in nature is actually restricted by unknown factors, or that the bacterium is pathogenic and recognizable only under limited, specific conditions, or that its presence cannot consistently be detected by existing culture methods, or that it is overlooked.

Several factors contribute to the lack of knowledge of the genus *Listeria*. These are an unawareness by bacteriologists of the bacterium, which often results in its being discarded as a contaminating diphtheroid; the difficulty of isolating it from certain animal tissues, with the consequence that the culture is reported as negative; the misconception that infection with *L. monocytogenes* is rare; the erroneous belief that all listeric infections are highly acute; the inability to reproduce experimentally some of the diseases with which it has been associated; and the failure to determine its natural reservoirs.

Still undetermined are the carriers that distribute and perpetuate the disease, and the factors that determine that in certain species infection usually results in encephalitis, while in others infection is most often manifested by meningitis, septicemia, abortion, or some other disorder. Also undetermined is the possible role of *L. monocytogenes* in syndromes such as infectious mononucleosis, habitual abortion in women, chronic mental disorders of children and adults, chronic liver disorders, and papular skin lesions. It even has been suggested that it may play a part in multiple sclerosis (105).

An increase in published case reports indicating *L. monocytogenes* as an important cause of deaths in the human newborn has stimulated renewed interest in the organism. A review of the literature related to the bacterium and the many diseases with which it has been associated might incite a further awareness of *L. monocytogenes* as a pathogen, dispel the persistent notion that listeric infections are rare and always acute, present a complete picture of an often neglected bacterium that seldom rates more than two or three pages in even the most recent textbooks of bacteriology, and indicate areas for further investigation that may lead eventually to closing the numerous gaps in knowledge of the epidemiology and pathogenesis of this much misunderstood bacterial infection. Several years ago, Murray presented for the Flavelle Medallist's Address an interesting parody entitled "The Story of Listerella" in which he pointed out some of the intriguing characteristics of the neglected and ignored bacterium which may yet emerge with a "golden slipper" (165). The present review is an attempt to present this Cinderella among the pathogenic bacteria.

HISTORY

Although as early as 1891, Hayem in France and in 1893 Henle in Germany observed gram-positive rods in tissue sections from patients who died of disease that, in retrospect, was almost certainly listeric infection, Hülphers (107), a Swedish worker, appears to have been first to record an encounter with the organism now known as *L. monocytogenes*. He isolated an organism which he called *Bacillus hepatis* from the necrotic foci in the liver of a rabbit in 1911. His description of the organism corresponds very closely to that of *L. monocytogenes*. The minor epidemic of meningitis reported by Atkinson (8) also may have been caused by this bacterium. These reports, and many others (136, 235), indicate that infections with members of this genus were seen prior to its first description in 1926 by Murray et al. (164). The latter isolated the bacterium from the liver of sick rabbits and guinea pigs and named it *Bacterium monocytogenes*. The following year, Pirie (201) isolated an identical bacterium from the liver of several gerbilles (*Iatera lobenquiae*), known as the African jumping mouse. He named it *Listerella hepatolytica*.

The disease now known as listeriosis was seen in sheep in Germany as early as 1925 according to Cohrs, and Matthews (155) in this country in 1928 reported an outbreak of encephalitis of unknown etiology in cattle, which very likely was listeriosis. Although Pletneva and Stiksova (204) claim the isolation of *L. monocytogenes*, then referred to as "X bacillus," from pigs in the Soviet Union in

1924 (in fact, one Russian author puts it as far back as the late 1800's), Gill (71), in New Zealand, generally is credited with the first isolation of *L. monocytogenes* from domesticated farm animals. In 1929, he observed a disease among sheep in Wales which he called "circling disease," a name still often applied to listeric encephalitis of ruminants. Two years later he succeeded in isolating a bacterium from the brain of affected animals and established the relationship between it and the disease. However, six more years passed before the true identity of the bacterium was determined. Since that time, infections with *L. monocytogenes* have become so widespread that today they are of not only considerable economic importance among the diseases of domesticated animals, but also are known to affect a wide variety of feral animals and birds that may have contact with them.

The first confirmed report of listeric infection in man was made by Nyfeldt (174) in 1929. He isolated the bacterium from three patients with an infectious mononucleosis-like disease. In this country, Burn (32) established *L. monocytogenes* as a cause of infection in the perinatal period in 1933. He encountered two more identical cases the following year, and a case of meningitis in an adult. Graham et al. (75) found that the bacterium could cause abortion in cattle, and Poppensiek (206) recorded the same condition in sheep; Biester and Schwarte (21, 22, 23) isolated *L. monocytogenes* from sheep, cows, and pigs in Iowa; Cole (86) in New York and Hoffman and Lenarz (86) in California encountered listeric septicemia in chickens. In spite of the confusion of war, Paterson (193), in England, recorded listeric abortion in sheep and Pallaske (86) found the bacterium among sheep and chickens in Germany. Schoop (231) isolated it from rabbits raised for food for German soldiers, while Krage (133) found it in a foal. In France, Cotoni (40) confirmed a case of meningitis in man reported by Dumont and Cotoni in 1921 as actually due to *L. monocytogenes*, and Forgeot et al. (65) established the presence of the disease in sheep. In the Netherlands, Kapsenberg (122) published his observations on four cases of meningitis in children and adults, and 2 years later Jansen and van den Hurk (114) found the bacterium in goats. During World War II occupation of Norway, Grini (77) observed encephalitis in sheep and septicemia in a foal, while in neutral Sweden Wramby (285) isolated the bacterium from chicken, cow's milk, and a dog, and Lilleengen (86) isolated it from a wood grouse. Behind the fighting front in the Soviet Union, Gudkova and Sacharov (93) studied an infectious mononucleosis-like disease characterized by high agglutinating titers against *L. monocytogenes* and occasional isolation of the bacterium. Both Fischer (62) and Savino (228) in South America recorded the isolation of the bacterium from cases of meningoencephalitis of man, and Macchiavello (151) recovered it from a wild rat. There were many other reports during the war years.

These reports produced perhaps the greatest concentration of publications on listeric infection in animals of any period. However, it was not until the mid-1950's that *L. monocytogenes* really passed from the awkward and unnoticed adolescent among the pathogenic bacteria into an exciting, sometimes coy, almost flirtatious bacterium—one that enticed and captivated and seemed to be everywhere. Yet, when the bacteriologist reached out to isolate it, it often eluded his culture. And so its proper place in human and veterinary medicine remains a riddle, but with an increased awareness of the disease stimulated by the ever-mounting number of case reports, it may eventually be found to be of far greater importance than previously suspected.

NOMENCLATURE

Justification for the Term "Listeric"

In general, the various susceptible animal groups show a somewhat characteristic clinical picture when infected by *L. monocytogenes*. These include: localized encephalitis or meningoencephalitis in ruminants; septicemia with a more or less conspicuous focal hepatic necrosis, but without involvement of the brain, in monogastric animals and young ruminants before the rumen is functional; septicemia with associated meningitis or meningo-encephalitis in man; and septicemia with myocardial degeneration in fowls. Abortion or other perinatal infections apparently can occur in all mammals and man. Since exceptions to the above generalities are recorded for all groups except young ruminants, and since infection shows such a wide variety of clinical manifestations, the terms "listeriosis," "listeriasis," and several others in rare use are meaningless when not clearly defined. For these reasons, these words are avoided in this review and replaced by "listeric" used as an adjective modifying the specific disease process.

Accepted Name of the Bacterium

L. monocytogenes is a member of the Family *Corynebacteriaceae*, order *Eubacteriales*. Until 1940, there was considerable confusion in the nomenclature of *L. monocytogenes* due to the interchange of the generic names *Listerella* and *Listeria*. Pirie (201), in 1927, chose *Listerella* as the generic name in honor of Lord Lister, the

well-known pioneer in the field of bacteriology. However, this name had already been applied to a group of slime molds (*Mycetozoaire*) by Jahn in 1906. The generic name *Bacterium* as applied by Murray et al. (164) was undesirable because the bacterium does not possess the characteristics of this genus. The resolution of the Committee on Nomenclature, Third International Congress for Microbiology, New York, 1939, that in all duplications of generic names only the one first applied should be considered valid, invalidated the generic name proposed by Pirie, and he suggested the name *Listeria* in 1940 (202). *Listeria* was adopted in the Sixth Edition of *Bergey's Manual of Determinative Bacteriology* and approved by the Judicial Commission on Bacteriological Nomenclature and Taxonomy (Opinion 12). Hence, there was conservation of *Listeria*, Pirie (1940) as a generic name in bacteriology (Intern. Bull. Bacteriol. Nomencl. Taxon. **4**:150–151, 1954), and it is now the official generic name.

The species name *monocytogenes* suggested by Murray et al. (164) is derived from the fact that large numbers of monocytes are often found in the peripheral blood of some naturally and artificially infected monogastric animals, although not in ruminants. It remains the only species within the genus. Thus, the official name today is *Listeria monocytogenes*, Pirie.

Synonyms recorded in the older literature include: *Bacterium hepatis* Hülphers, 1911 (107); *Bacterium monocytogenes* Murray et al., 1926 (164); *Listerella hepatolytica* Pirie, 1927 (201); *Listerella monocytogenes hominis* Nyfeldt, 1932; *Corynebacterium parvulum* Schultz et al., 1934; *Listerella ovis* Gill, 1937 (71); *Corynebacterium infantisepticum* Potel, 1950 (209); and *Listeria infantiseptica* Potel, 1952. Some early authors employed such names as *Listerella bovina*, *L. gallinarium*, *L. cuniculi*, *L. suis*, and *L. gerbilli*. These are seldom encountered today.

CHARACTERIZATION OF THE BACTERIUM

Morphology

The smooth, pathogenic form *L. monocytogenes* is a small gram-positive, nonsporeforming, non-acid fast, diphtheroid-like rod with rounded ends measuring 1.0 to 2.0 μ by 0.5 μ. It is usually considered noncapsulated, but recent immunocytological and immune electron microscopic studies by Smith et al. (246) at the Armed Forces Institute of Pathology revealed a mucopolysaccharide capsule to be present. It is easily confused with members of the genus *Corynebacterium*, and no doubt has been discarded often as a "contaminating diphtheroid." Frequently there may be long rods measuring 5.0 to 7.0 μ or chains composed of three to five or more cells. Smears from 15- to 24-hr-old colonies show typical diphtheroid palisade formation with some V and Y forms, and a few diplococcoid forms or actual cocci. Coccoid forms are encountered most often in smears from infected tissue or broth cultures but are rare in smears from colonies. This form leads to much confusion with the streptococci. In 24- to 36-hr-old colonies, cells are definitely gram-positive, but examination of older cultures or broth cultures often reveals gram-negative cells. Poorly stained smears may resemble *Haemophilus influenzae*, and this has been a source of some confusion.

Studies of the fine structure of *L. monocytogenes* as revealed in electron micrographs of thin sections of cells of the bacterium were reported in 1963 by Edwards and Stevens (56*a*), Grund (92*a*), Kawata (124*b*), and North (171*a*). These papers are well illustrated with electron micrographs. Within the cell wall, the plasma membrane is complex, involving more than the three standard (dense, light, and dense) layers usually attributed to a unit membrane. In *L. monocytogenes*, the plasma membrane has three dense layers, each varying in width from 15 to 35 A, which alternate with two light zones (the bridged and unbridged layers), each with an average thickness of 30 A. The plasma membrane is continuous with a system of internal membranes variable in size and shape, located in the cytoplasm and nuclear area. They may be simple invaginations, spiraled structures, or complex organelles of various appearances. The cytoplasm may be packed with dense granules less than 100 A in diameter. The nuclear apparatus has the same general features found in other bacteria. It has a low density as compared with the cytoplasm, containing fibrils 25 to 50 A in diameter, appearing as rows of beads or twisted filaments. The fibrils may represent sections of a long deoxyribonucleic acid molecule, coiled and unbroken inside the nucleoid, as in a ball of yarn.

Cultural Characteristics

Cultivation. Although isolation of *L. monocytogenes* may be difficult at times, after initial growth on artificial media it usually grows well on most commonly employed bacterial media. Tryptose Agar (Difco) is an excellent substrate for cultivation and preservation of the bacterium. This medium has the additional advantage of being clear and colorless. When cultures grown on a colorless medium are viewed with a binocular scanning microscope, or even a hand lens mounted on a laboratory tripod (15), with the use of obliquely transmitted illumination as de-

scribed by Henry (102), the colonies have a finely textured surface and are distinctive blue-green (78–80, 82, 83). The appearance is so characteristic that, with a little practice, colonies can be identified quickly even in contaminated material (15, 82, 84, 88, 97, 98, 152). After 24 hr of incubation at 37 C, the colonies are round, translucent, slightly raised with a finely textured surface, have an entire margin, are bluish-gray by normal illumination, and are watery in consistency. They range from 0.3 to 1.5 mm in diameter depending on colony population density. After 5 to 10 days, well-separated colonies may reach 3 to 5 mm or larger in diameter, be slightly umbonate, rubbery, and often show evidence of dissociation (83). Colonies on blood-agar are similar in appearance and may have a narrow zone of β hemolysis. This is especially true for freshly isolated cultures. Hemolysis can be demonstrated with sheep, cow, horse, rabbit, or human blood. Certain strains may exhibit hemolysis on rabbit blood-agar but not on sheep blood-agar. Some sheep may have antibodies against *L. monocytogenes* perhaps as a result of inapparent infections.

In fluid media, *L. monocytogenes* produces faint clouding in 18 to 24 hr, depending on size and age of inoculum. Very small inocula may fail to grow. After several days, a thick, sticky, slimy precipitate forms which, when agitated, produces a climbing corkscrew effect. Continued incubation produces a precipitate so tenacious that it is virtually impossible to disintegrate it. Growth usually is increased by the presence of a fermentable sugar, particularly glucose, the clouding is more dense, and after several days of incubation, in contrast to that described above, a flocculent precipitate appears. Agitation greatly enhances growth in complex media (66, 125) but inhibits it in defined media (43, 66).

L. monocytogenes grows best in neutral to slightly alkaline medium. It will grow at *p*H as high as 9.6 but usually dies at *p*H lower than 5.6. For this reason, it is often impossible to make viable transfers from cultures used in fermentation studies, especially if the reaction has been strongly positive.

All cultures have a characteristic sour, buttermilk-like odor which is more marked in cultures grown on solid media.

L. monocytogenes grows from 3 C to about 45 C, with optimal growth between 30 and 37 C. At 37 C, the growth curve peak is reached in 16 to 18 hr. Although growth is slow at 3 to 4 C, turbidity in broth or growth on solid medium can be observed in 5 to 8 days. At 6 C, the log phase is reached in 10 to 11 days (281). These cultures are highly motile, possess well-developed flagella, and are pathogenic for laboratory animals (235). Avery (89) and Laymann (146) found cultures grown at 4 C to be more pathogenic than those grown at 37 C.

Motility and flagellation. L. monocytogenes has characteristic tumbling motility, best demonstrated in cultures incubated at "room temperature." Tests for motility should always be carried out at room temperature. Failure to do so has led to much confusion regarding motility and flagellation, since most cultures incubated at 37 C are, at best, only sluggishly motile, and possess few, if any, flagella. Seeliger (235) studied a considerable number of supposedly nonmotile cultures and found all to be motile when incubated at 20 C. Although most authors report marked motility at 4 C, Kleikamp (129) saw only sluggish motility in cultures grown at 4 C unless 10 to 20% CO_2 was added to the atmosphere. She also reported increased motility at 37 C for cultures grown in an atmosphere of 20% CO_2. Increased CO_2 had no effect on cultures incubated at room temperature.

Any method commonly employed for demonstration of motility may be applied to *L. monocytogenes*. Stab cultures into semisolid motility medium produce typical, inverted "pine tree" effects. Seeliger (235) favored use of a Vahlne U tube. One arm is inoculated with culture and incubated at room temperature. Progress of motile cultures can be followed by clouding of the otherwise clear semisolid medium. Under these conditions, maximal growth occurs approximately 5 mm below the agar surface of both arms, indicating that *L. monocytogenes* grows best in a slightly anaerobic environment.

Comparatively few studies have been devoted to the flagellation of *L. monocytogenes*. Although several early investigators reported a single polar flagellum (32, 71, 107), Paterson (194) first found peritrichous flagella and suggested that the bacterium might be monoflagellated at 37 C. Griffin and Robbins (92), using Gray's flagella stain, found that 90% of the cells incubated at 37 C were nonflagellate whereas only 20% of those incubated at room temperature were nonflagellate. More than 50% of cells grown at room temperature were either monotrichous or biflagellate; the remainder, tri- or tetraflagellate. When incubated at 37 C, no cells were tetraflagellate and less than 1% were triflagellate. They found no evidence of polar flagellation. Gray (223), in similar studies with an electron microscope, obtained essentially identical results, with the exception that the majority of cells incubated at room temperature were triflagellate. Regardless of the number of flagella, the point of

attachment appeared to be subterminal, but never polar. Csontos et al. (42) reported no flagella in electron micrographs of four strains incubated at 37 C and only a few flagella when the same cultures were incubated at room temperature.

According to Roots (221), pure flagella of *L. monocytogenes* are composed of protein and contain no O antigen.

Tightly coiled or spiral-like flagella have been observed in some cultures of *L. monocytogenes*. Leifson and Palen (148) and Hartwigk and Grund (96) feel this to be a definite characteristic of certain strains. Gray (223) contests this view, since he observed considerable alteration of flagellar configuration resulting from the manner of preparation and mounting on the microscope grids. Leifson and Palen (148), in a study of 81 stock cultures stained with Leifson's flagella stain, found the average mean wavelength of the flagella to be 2.01 $\mu \pm 0.01 \mu$ with an amplitude of 0.48 $\mu \pm 0.007 \mu$. These authors and Griffin and Robbins (92) both reported the existence of nonmotile, flagellated strains at 37 C. However, Seeliger (235) found all of these strains to be motile under proper conditions and to contain well-developed H antigens. Electron micrographs and further studies by Fuhs and Seeliger (66*b*) on H antigen production demonstrate the development of flagellation in a "nonflagellated" strain when cultivated between 20 to 30 C. The available information suggests that all smooth cultures of *L. monocytogenes* possess flagella and are motile, but that motility may not be evident under certain cultivation methods.

Hartwigk and Grund (96) noted small, dense, round particles lying in or along the exterior cell wall of cultures examined with an electron microscope. The significance of these has not been determined. It is conceivable that they might represent phage particles as reported by Sword and Pickett (260), especially since some were found at a rather considerable distance from the cell. According to Gray (223), they appear to be artifacts not removed by washing. He saw similar particles only in specimens that were not thoroughly washed.

Hemolysin production. L. monocytogenes produces a soluble, filterable hemolysin capable of attacking most mammalian erythrocytes. Hemolysis on blood plates is of the β type and is characterized by a very narrow zone. It is most pronounced in freshly isolated cultures and may be completely absent in old, laboratory-maintained strains. Kleikamp (129) found no increase in hemolysin production by increasing the CO_2 content of cultures incubated at 37 C or room temperature, but there appeared to be slight enhancement at 4 C. However, hemolysin production was very poor at 4 C and, in any atmosphere, the zone seldom extended beyond the periphery of the colony.

Girard et al. (74*a*) found that *L. monocytogenes* produced a soluble hemolysin which could be precipitated from culture filtrates by 60% saturated $(NH_4)_2SO_4$ at 5 C. The hemolytic activity was in the euglobulin fraction. It was protein in nature and migrated electrophoretically as a γ-type globulin. Attempts were made to measure antihemolysins by a procedure adapted from the antistreptolysin O method. Njoku-Obi et al. (169*a*) questioned the value of an antihemolysis test in the serological diagnosis of listeric infections because of the high antihemolytic activity of normal sera from several species. Jenkins et al. (115*a*) further purified the hemolysin after ammonium sulfate precipitation by a series of adsorptions and elutions from calcium phosphate gels. Cysteine, sodium hydrosulfite, and a number of reducing agents markedly increased the potency of purified hemolysin. Many of the actions of purified hemolysin seemed to parallel those of streptolysin O. Rogul and Alexander (218*b*) found listeric hemolysins to be similar to those described for oxygen-labile hemolysins.

Toxin production. Since the earliest description of lesions resulting from infection with *L. monocytogenes*, the toxic nature of the alteration has been emphasized. Although many attempts have been made to demonstrate either an endo- or exotoxin, none was successful. Stanley (253) produced death of rabbits in a few hours after injection of a polysaccharide fraction of the bacterium, but did not mention lesions in these animals. The same fraction, given in amounts lower than the LD_{50}, produced neutrophilic leukocytosis preceded by leukopenia. Several other investigators (124, 197, 235) found that fractions or extracts of the bacterium enhanced susceptibility to listeric infection. Smith et al. (89, 248) inconsistently produced lesions in rabbit spleen cultures with sterile filtrates of the bacterium which appeared to be identical to those produced by the living culture (248), but the responsible factor was never isolated. Recently, Liu and Bates (150) succeeded in demonstrating toxin in sterile filtrates of cultures grown in Trypticase Soy Broth (BBL). The toxin was capable of producing hemorrhagic lesions within 3 hr when inoculated intracutaneously in rabbits. Within 18 hr, the lesion became necrotic and similar in nature to those produced by living culture (83). The filtrates also killed mice within 24 to 48 hr after intraperitoneal injection. At necropsy, there was focal necrosis similar to that

resulting from living cultures. The toxin was inactivated by heating at 70 C for 30 min; it was not precipitated with 30% saturated ammonium sulfate, but was precipitated by three volumes of ethyl alcohol at −5 C. The agent has not been further characterized, but the report appears to be the first published demonstration of toxin produced by *L. monocytogenes*.

Robinson and Njoku-Obi (218*a*) prepared a toxic polysaccharide from *L. monocytogenes* cells grown on Trypticase Soy Agar by treatment of the cells with phenol and further fractionation with absolute alcohol, sodium acetate, and acetic acid. The purified material produced skin reactions in rabbits. McIlwain et al. (156*a*) isolated three proteinaceous fractions from disrupted cells of the bacterium. One toxic cellular protein fraction produced abnormalities in the electrocardiogram tracings, particularly an increase in the T wave, and distortion of the QRS complex when injected into rabbits. The animals also had increased respiration, elevated body temperature, hyperglycemia followed by hypoglycemia, and blood sugar depletion in rabbits which died. Pyruvic and lactic acid concentrations increased. There was a significant increase in monocytes within 48 hr after injection. Rabbits injected with viable *L. monocytogenes* developed monocytosis and elevated body temperatures, but after a greater time than similar animals injected with the toxic cellular protein fraction.

Fermentation and Biochemical Properties

Fermentation pattern. As early as 1939, Julianelle (118) pointed out that the similarity of saccharolytic reactions of various strains of *L. monocytogenes* did not form a sufficient basis for classification. Fourteen years later, Murray (165) echoed this finding in commenting on the striking consistency of the bacterium's biochemical characteristics in spite of the astonishing variety of susceptible hosts and extensive distribution throughout the world. Unfortunately, the numerous reports of fermentation and biochemical reactions published during and after that interim present an almost equal number of conflicts. Many apparent discrepancies may reflect differences in basal medium, concentration of fermentable substance, indicator employed, temperature and time of incubation, and method employed for biochemical determinations. In support of this, Gray (78, 89) grew 51 strains of *L. monocytogenes* from animal and human sources, representing serological types 1 and 4b, and consistently produced blackening of lead acetate strips in a medium composed of liver infusion, Witte's peptone, sodium chloride, and distilled water. Since there were no previous reports of H_2S production by *L. monocytogenes*, several other accepted media for production of H_2S were inoculated with cultures taken at random from this group. H_2S was not produced in any of these media. Also, all of 161 cultures studied by King (89) produced H_2S on lead acetate paper over triple-sugar iron slants but not in the butt. In contrast, Seeliger (235) reported no H_2S production in 408 cultures. Similarly, he found that choice of indicator had a marked effect on results of fermentation studies. When the indicator used had a turning point very close to neutral, several sugars showed evidence of fermentation. Other indicators with a somewhat lower color change point showed no reaction with these same sugars. Since *L. monocytogenes* has been studied in laboratories throughout the world with the use of innumerable different substrates, it is not surprising that contradictory reports have appeared.

For various reasons, reactions given as "typical" are based largely on the findings of Seeliger (235), King (89), and Gray (89), all of whom studied relatively large numbers (100 to 400) of cultures under essentially consistent and almost identical conditions. In 24 hr at 37 C, acid but no gas is produced in glucose, levulose, trehalose, and salacin. Acid production proceeds more slowly, 3 to 10 days, or it may be completely absent in arabinose, galactose, lactose, maltose, rhamnose, sucrose, dextrin, sorbitol, glycerol, esculin, and melezitose. Seeliger (235) claimed xylose fermentation by some strains, but this was contradicted by King (89) and Gray (89) on the basis of studies on some 300 cultures. Recently, King (89) reported that xylose was fermented only when it was autoclaved in the medium. Fermentation has not been reported for raffinose, inulin, inositol, dulcitol, adonitol, and mannitol.

Distinct strain differences in ability of *L. monocytogenes* to ferment certain sugars, particularly sucrose, lactose, and melezitose is well established. Gudkova and Sacharov (93), of the Soviet Union, claimed that strains of *L. monocytogenes* passed through brain tissue lose their ability to ferment sucrose and lactose and that the fermentation of these substances may be an index of affinity for nerve tissue. This observation is interesting, although unconfirmed.

Harvey and Faber (99) suggested the fermentation of melezitose as a basis for division into biotypes closely resembling Paterson's (194) serological types. In an attempt to confirm this observation, Seeliger (235) studied 120 cultures isolated from man and animals, and representing all continents. He found it impossible to type the bacterium on this basis. Even cultures from the

same immediate locality showed variation in ability to ferment this sugar. However, the cultures tended to fall into a poorly defined pattern. Of 45 type 4 cultures, 41 developed acid within 2 to 4 days, whereas, of 72 type 1 cultures, 48 were negative.

Biochemical properties. Biochemically, *L. monocytogenes* does not reduce nitrate or produce indole. As noted above, most authors report no H_2S production, but it may be produced under appropriate conditions. Gelatin and coagulated serum are not liquefied, starch and urea are not hydrolyzed, and ammonia is produced from the hydrolysis of arginine. The Voges-Proskauer test is positive. The methyl red test is usually positive, but the choice of peptone employed may play an important part in the result. Litmus milk is acidified slowly and eventually decolorized, but not coagulated.

Nutritional Requirements and Metabolism

There is a paucity of information on the nutritional requirements and metabolic pathways followed by *L. monocytogenes*. It is well established that relatively simple media will support growth of the bacterium, but few efforts have been made to determine the exact growth requirements. Porter and Pelczar (208) as early as 1941 made the first attempt in this direction and obtained good growth in a medium containing riboflavine, biotin, hemin, and protein hydrolysate in addition to the usual salts and sugar. One strain grew inconsistently with riboflavine as sole growth factor. This observation was confirmed a year later by Hutner (109), who suggested the use of *L. monocytogenes* for riboflavine assay. These studies were extended with the collaboration of Cury and co-workers (43), and led to the development of a synthetic medium containing 19 essential amino acids, dextrose, and mineral salts, and supplemented with riboflavine, biotin, thiamine, and thioctic acid. With the omission of any factor, the medium failed to support growth. Welshimer (281*a*) found that all strains failed to grow for more than one or two passages in the absence of riboflavine, biotin, or thioctic acid. The thioctic acid requirement and the antagonistic effect of its analogue, 8-methylthioctic acid, could be modified by altering the thiamine concentration. The addition of a variety of other vitamins and nutrients did not enhance growth. These studies also confirmed an earlier observation of Hutner (109) that "vitamin-free" casein hydrolysate contained a nonessential factor which greatly stimulated growth. A similar factor was also found in human urine. These authors also suggested that *L. monocytogenes* might be employed as a tool for the quantitative measurement of thioctic acid.

More recently Friedman et al. (66) successfully cultured *L. monocytogenes* in a defined medium composed of 21 amino acids, salt, glucose, and vitamins, but only if incubated in stationary condition, confirming the finding of Cury et al. (43). Agitated cultures required substitution of an enzymatic digest of protein for the synthetic amino acids, suggesting a requirement for peptides and possibly other growth factors for aerobic cultivation. Synthetic peptides did not enhance growth with shaking. However, as Keeler and Gray (125) also found, agitation greatly enhanced growth in a complex medium. Valine, leucine, isoleucine, and organic sulfur were essential, but sulfur could be replaced by cysteine, thioglycolate, or cystine. A minimum of nine amino acids, six in addition to three essential, supported growth of some cultures. The requirement for lipoic acid as suggested by Cury et al. (43) could not be confirmed by these authors. In addition to the organic compounds, Mg^{++} and PO_4^{-3} were required while K^+ was either required or, at least, greatly stimulated growth. Larson et al. (144*b*) found one of 128 strains studied which required either isobutyric or 2-methylbutyric acid for growth. The production of phosphatase, lipase, and phospholipase by cultures of *L. monocytogenes* was described by Luppi and co-workers (150*a*, *b*, *c*).

Miller and Silverman (158) found that carbohydrates were essential for good growth of *L. monocytogenes*. Glucose could not be replaced as an energy and carbon source by gluconate, xylose, arabinose, or ribose. Resting-cell fermentation experiments indicated that two three-carbon compounds were formed per glucose moiety, suggesting that glucose degradation was independent of the degree of aeration. Soluble extracts of the bacterium contained enzymes of the Embden-Meyerhof pathway; only glucose-6-phosphate and 6-phosphogluconate dehydrogenase of the shunt pathway were present, but not the 6-phosphogluconate splitting enzyme of Entner and Doudoroff. These authors suggested that *L. monocytogenes* might be closely related to the *Lactobacteriaceae*, since principally lactic acid was produced from carbohydrates. This relationship was also stressed by Cury et al. (43).

In somewhat similar studies, Kolb and Seidel (130) calculated the oxygen consumption of washed cells of *L. monocytogenes* during a 5-hr period to range from 19 to 35 μliters of oxygen per 0.05 mg of nitrogen at 22 C to 105 to 118 μliters at 45 C, indicating a relatively rapid respiration rate. Glucose, lactose, fructose, maltose,

and saccharose increased the respiration rate in that order. Respiration was considerably increased by the addition of malic, pyruvic, and α-ketoglutaric acids. When these studies were extended to include the effect of various antibiotics on respiration, penicillin and hostacyclin produced the greatest inhibition of respiration.

In the experiments of Friedman and Kautter (66*a*) and Friedman and Alm (65*b*), *L. monocytogenes* was only one-fourth to one-ninth as virulent for mice by the respiratory route when grown in 1% glucose as when grown in 0.2 to 0.6% glucose. Increased glucose concentration was associated with a decrease in dehydrogenase and catalase activities.

That smooth pathogenic and some, but not all, nonpathogenic rough cultures of *L. monocytogenes* contained dehydrogenases capable of reducing 2,3,5-triphenyltetrazolium chloride to colorless formazans was reported by Gray et al. (83). Dias and da Silva (50) observed small intracellular granules located at the poles or center of cells in electron micrographs of cultures exposed to this salt. These granules were thought to be related to mitochondria or to be involved in cellular metabolism, and were absent in cells not exposed to the salt. Krcmery (135) used this tetrazolium salt to study the dehydrogenases involved in the degradation of glucose and pyruvic acid, using 35 strains of *L. monocytogenes*. Unfortunately, results are incomplete and no conclusions can be drawn.

These several somewhat limited studies emphasize that much unfinished work must be completed for a better understanding of the nutritional requirements and metabolism of *L. monocytogenes*. Little has been done to investigate the 10-year-old suggestion of Özgen that methane-propane mixture may enhance growth of the bacterium or to resolve the conflict of whether *L. monocytogenes* favors a slightly anaerobic environment, as often suggested, or whether optimal growth is obtained under strictly aerobic conditions. The determination of some of these still unknown factors, particularly essential growth requirements, might possibly open a pathway around the perplexing problems sometimes encountered in attempting to make initial isolations from infected material as referred to under METHODS FOR ISOLATION.

Colonial Dissociation

Cultures of *L. monocytogenes* incubated for several days often have a marked tendency to dissociate. This has been observed and mentioned by numerous investigators since the earliest studies dealing with the bacterium. Yet, in spite of this tendency, few efforts have been directed toward thorough study of the dissociation pattern or to exploit the possible use of variant cultures as potential vaccines.

It is somewhat amusing that a characteristic as conspicuous as colonial dissociation is one of the few things about *L. monocytogenes* not included in the first report by Murray et al. (164). This omission allowed Anton (5) to make first mention of rough forms, and, because long filaments developed in some old cultures, he speculated that the bacterium might actually be a fungus. Most early investigators mentioned only two or three different colonial forms which, in general, were designated "smooth," "intermediate," and "rough." Halos of secondary growth around some colonies were reported occasionally. Depending on the author, the various nonsmooth cultures had marked differences in either loss of hemolyzing power and differences in colony or cell morphology and pathogenicity, or both (83). Lowering the *p*H greatly enhanced development of rough forms (13, 43, 89). It must be recognized that standardized media composed of carefully controlled ingredients were not available until recently, and it is difficult to evaluate some older reports since they may actually represent differences due to media rather than true differences in colonial types. Almost all investigators who reported the presence of R forms called attention to the filamentous nature of the cells, and many pointed out the striking resemblance to *Erysipelothrix insidiosa*. Potel (83) suggested that these filaments result from failure of individual cells to separate after division.

Hunter et al. (108) appear to be the only group who attempted to produce artificial mutants. They exposed a culture of *L. monocytogenes* to β particles from radioactive phosphorus and detected 21 colonial variants. Only a few of these variants differed essentially from the parent strain in fermentative ability, biochemical reactions, or antigenic structure. Five variants showed greatly decreased pathogenicity for embryonating chicken eggs and white mice. No mention was made of the variant's ability to produce monocytosis in infected mice. The five variants also failed to hemolyze horse blood, to ferment lactose and melezitose, and to produce methyl red and Voges-Proskauer reactions.

Leifson and Palen (148), in a study of flagellar morphology and motility of 81 stock strains of *L. monocytogenes*, referred to the colonies as "spreading" or "nonspreading." By culturing the spreading growth, several types of mutants were obtained in pure culture, but these were not further characterized.

By means of Henry illumination (102), Gray et al. (83) detected at least 20 different colonial forms in 20 cultures of *L. monocytogenes* isolated from man and animals and grown on "dry" tryptose agar plates. The naturally occurring S culture gave rise directly or indirectly through intermediates to 19 types designated as nonsmooth or rough. These types were characterized by colonial forms which differed in texture, consistency, configuration, opacity, or color from the S type. A few R colonies were pink after the first 24 hr of incubation but changed to the characteristic blue-green after several days of additional incubation. Only one pink colony failed to show the color change. Cells ranged from short gram-positive rods in S colonies to rather long, thick, curved gram-variable rods or extremely long, slender gram-positive filaments in R colonies. Only the S, two intermediates, and one R form were pathogenic for rabbits when inoculated intravenously, subcutaneously, or instilled into the conjunctival sac. However, nonpathogenic R cultures produced marked monocytosis after intravenous or subcutaneous exposure. Six R forms could be maintained in stable form. On the basis of fermentative and biochemical reactions, R types were essentially indistinguishable from the parent S type. However, there were differences in physiological functions as revealed by complete or partial inability of a number of R cultures to reduce 2,3,5-triphenyltetrazolium chloride to colored formazans, a reaction accomplished readily by S cultures. This reaction can be employed as a convenient method for distinguishing some R cultures which otherwise may appear very similar to one another. Limited studies on antigenic structure showed that some R types shared common antigens with the S type while others did not. As with other bacteria, R cultures showed a marked tendency toward autoagglutination.

Some cultures dissociated more rapidly than others. Some tended to produce a predominance of a particular R type, some showed predominance of a different type, and other displayed almost no tendency toward dissociation aside from development of a few intermediates. The significance of this dissociation can only be conjectured. Whether cultures isolated from a particular disease process, or with certain antigenic configuration, have a distinctive pattern has not been determined. This possibility should be an enticing field for further investigation and could lead to productive results.

There is considerable disagreement as to whether R cultures of *L. monocytogenes* will revert to S. Seeliger and Linzenmeier (235) failed to find evidence of R to S reversion even after 18 months of observation. In contrast, Potel (209) claimed reversion of R cultures of reduced pathogenicity to S cultures of enhanced pathogenicity by repeated transfer through broth or on blood-agar plates, and also by animal passage. Dedié (45) also reported reversion to S of R cultures passed through embryonating chicken eggs. However, three rough cultures studied by Paterson (195) were not pathogenic for chicken embryos, and he did not mention reversion to S. Although Gray et al. (83) often isolated a few S colonies from R cultures maintained for long periods in tryptose broth containing 2,3,5-triphenyltetrazolium chloride, or from the small localized necrotic lesions that developed in some rabbits after simultaneous subcutaneous injection of R cultures and hyaluronidase, they interpreted this as an indication that all R cultures contained a few S cells, rather than true R to S reversion.

Most reports agree that true R cultures are not pathogenic. However, monogastric animals exposed to R cultures, or others designated "nonpathogenic," usually produced monocytosis without other clinical manifestations. Stanley (253), who isolated the monocyte-producing factor of *L. monocytogenes*, found that R cultures also contained the factor, but in much smaller quantity than S cultures.

At least two reported variants of *L. monocytogenes* are open to question. Sonir et al. (235) isolated a "nonpathogenic" strain from cooked beef. Seeliger (235) feels that this is a valid member of the genus *Listeria*, whereas Gray (89) could find few, if any, characteristics to justify inclusion in this genus. Orobinskii (185) in the USSR described a green pigment-producing variant. If this is a valid member, it is noteworthy that such a culture had not been encountered previously.

Rough colonies are occasionally seen in cultures isolated directly from infected material. However, there is no known report of the isolation of a pure R culture.

Few attempts have been made to exploit nonpathogenic R cultures in the development of potential vaccines. The meager evidence available is not encouraging and is discussed more fully under vaccines.

Antigenic Structure

Serological studies with *L. monocytogenes* were initiated by Seastone in 1935. However, Schultz et al. (232) and Julianelle (118) first suggested the presence of two distinct serological groups. Julianelle designated these groups as type I, or rodent strain, and type II, or ruminant strain.

TABLE 1. *Antigenic structure of Listeria monocytogenes*

Serotype	O factors	H factors	Antigen for absorption	Specific factor(s)
1a	I, II, (III)*	A, B	Type 3 O	I
1b	I, II, (III)	A, B, C	Type 1a H	C
2	I, II, (III)	B, D	Type 1 H	D
3a	II, (III), IV	A, B	Type 1 or 2 O	IV
3b	II, (III), IV	A, B, C	Type 1a H	C
4a	(III), (V), VII, IX	A, B, C	Type 4c O	IX
4b	(III), V, VI	A, B, C	Type 4a O	VI
4c	(III), V, VII	A, B, C	Type 4b O	VII
4ab	(III), V, VI, VII, IX	A, B, C	Type 4c O	VI, IX
4d	(III), VI, VIII	A, B, C	Type 4b O	VIII
4e	(III), V, VI, VIII, IX	A, B, C	Type 3 O	V, VI, VIII, IX

* Parentheses indicate irregularly occurring factors.

He postulated that the ultimate origin of strains isolated from infective material could be determined by serological methods. The almost simultaneous study of Paterson (194), using a much larger number of cultures than was available to Julianelle, showed this suggestion to be untrue. Subsequent studies throughout the world relating to the antigenic structure of *L. monocytogenes* now firmly establish that the serotype is in no way related to host species or disease process, and only slightly to geographical origin.

Paterson (194) divided the genus *Listeria* into four serological types on the basis of somatic (O) and flagellar (H) antigens. Types 1, 3, and 4 were differentiated on the basis of their O antigens, and type 2 contained a distinctive H factor. Although numerous cultures have been isolated from a wide variety of new sources during the 20 years since Paterson's report, only slight modifications have been made in his original scheme. It is a striking coincidence that among the 54 cultures available to Paterson, both types 2 and 3 were included. These types are so rare that they were not to be isolated again for almost 15 years, and fewer than 15 cultures of these types have been recognized. Seeliger (235) divided the original type 4 group into types 4a and 4b on the basis of somatic antigens, and Donker-Voet (52) further divided the group into types 4c, 4d, and 4e on the basis of somatic antigens. She also found slight differences in flagellar antigens of some type 1 and type 3 cultures, and designated these as type 1a and type 3a. The presently recognized serotypes with their antigenic formula and specific factor are given in Table 1.

A comparison of the two groups described by Julianelle and the four described by Paterson show Julianelle's rodent group to correspond to Paterson's type 1, while Julianelle's ruminant group corresponds to type 4. The origin of the cultures used by Julianelle gives a possible clue why he reached the conclusion he did. The rodent cultures were isolated from rabbits and guinea pigs in England by Murray et al. (164) and from a gerbille in South Africa by Pirie (201). Pirie's "ruminant" cultures originated in the USA. Recent serological typing of large numbers of cultures indicates type 1 (rodent) to be predominant in Europe and Africa, while type 4 (ruminant) is predominant in the USA (52, 89, 121, 128, 235). With the limited number of cultures at his disposal, it is readily apparent why he reached this erroneous conclusion.

Seeliger (89) encountered an interesting situation. When he began large-scale serological typing of cultures isolated in Germany in the early 1950's, type 1 was the predominant type. Gradually, an increasing number of new isolants proved to be type 4b. In 1958, 75% of 108 cultures isolated from man and 58% of 29 cultures from animals were type 1. By late 1960, for some still unexplained reason, most new isolants in Germany were type 4b. Although there is no explanation, intriguing speculation is invited. In 1955, several chinchillas were imported from the USA. Shortly after their introduction into a colony in Germany, there was a large outbreak of listeric abortion and septicemia (220). The cultures isolated were type 4b, strongly incriminating the imported animals as carriers and responsible for the disease. Almost all cultures isolated from chinchillas in the USA have been type 4b (89). Although substantial evidence indicates that many healthy human beings may be carriers, the possibility has been completely unconfirmed that the large number of Americans in military service in Germany and the numerous tourists who visit there each year may have introduced type 4b cultures. About 70% of some 600 cultures typed in the USA were type 4b (52,

89, 128). In the Scandinavian countries, with the exception of the original cultures isolated by Nyfeldt between 1929 and 1933 which were all type 3, type 1 occurred almost exclusively until the past few years. The apparent changing pattern of serological types in Europe offers a potentially rewarding challenge to immunologists.

Serology

Agglutination reaction. In general, little difficulty is encountered in preparing satisfactory antigens of *L. monocytogenes*. A highly motile culture is best for production of H antigen. Donker-Voet (52) recommends inoculating the culture into 0.5% agar in petri plates, incubating at room temperature, and selecting highly motile colonies. The cultures are then transferred into fluid medium or inoculated on "moist" agar surface. Incubation must be at 18 to 22 C to insure maximal flagella production. After 36 to 48 hr of incubation, formalin is added to give a final concentration of 0.25 to 0.30%. The suspension is shaken at 37 C for 24 to 48 hr. The antigen can be used for immunization of animals or for agglutination tests. If desired, the bacterial cells may be sedimented by centrifugation and resuspended in saline to the desired concentration.

Cultures for O antigens should be grown on "dry" solid medium and incubated at 37 C to discourage flagella production. After 24 to 36 hr of incubation, growth is washed off, suspended in saline, and boiled for 1 to 2 hr in a water bath. The suspension can be used for either animal immunization or agglutination tests. Although it seems quite simple, the fact is well established that O antigens of *L. monocytogenes* show marked tendency to autoagglutinate. A number of means to overcome this feature have been suggested. They include growth on media containing buffers, lowering salt concentration to 0.2%, washing cultures in distilled water, buffering the saline or other vehicles, ultrasonic or sonic oscillation, and growth on cellophane over an agar surface. Czwalina (44) compared the efficacy of a number of these antigens and found, as have many others (89, 235), that ultrasonic or sonic oscillation gives superior results. Reactions are clear and show well-defined end points. In fact, reactions can often be read after 2 hr of incubation in a water bath at 45 to 52 C, whereas other methods usually require holding overnight at 4 C. If no such equipment is available, the use of 0.2% salt concentration as recommended by Boekels (27) gave most satisfactory results. Füzi and Pillis (67) claimed excellent results with the cellophane over agar technique, but as far as is known this finding has not been evaluated by others.

Osebold et al. (185*a*) demonstrated a protein-like surface substance on *L. monocytogenes* which caused inagglutinability of some heat-killed antigens. Treatment with trypsin eliminated the inagglutinability and increased the sensitivity of somatic antigen preparations.

Although any preservative may be used with antigens of *L. monocytogenes*, phenol should be avoided since it tends to enhance autoagglutination.

The O and H agglutination test is best carried out by incubation in a water bath at 45 to 52 C for 2 hr followed by storage at 4 C overnight. After the tubes are removed from the refrigerator, they are allowed to sit at room temperature for approximately 30 min before reading.

The specific factors required for serological typing may be produced quite readily by any of the usual techniques for this purpose. Absorption should be repeated at least twice; incubation time, temperature, and conditions are the same as for the agglutination test. H antigen absorption may be somewhat more effective if initial incubation is carried out for 1 hr, at room temperature rather than in a water bath.

The relatively early series of studies by Robbins and Griffin (218) remains the only one directed toward an understanding of individual antigenic components (during antigen production) and subsequent immunization of hyperimmune animals. Killing by minimal heat, formaldehyde, chloramine-T, Merthiolate, or phenol had no adverse effect on the total capacity of *L. monocytogenes* to produce H antibodies. However, factor A was destroyed by chloramine-T, Merthiolate, and phenol, while factor C was slightly affected adversely by Merthiolate. With the exception of factor III, O factors were not affected by prolonged boiling or high concentrations of alcohol. Factor III behaved erratically after treatment with both heat and alcohol but did not lose its identity.

During immunization, H factors A, B, and D appeared early, but factor A tended to decrease toward the end of the immunization period. Although factor C was late in appearing, it developed rapidly during the later stage of immunization. Antibody production against O factors was more erratic, particularly that against factor III, which remained low during the early stages but rose to relatively high titer (1:640) after the fifth week. Seeliger (236) and Mannweiler (223) each observed that, both in animals hyperimmunized with OH antigen and in antibody response to naturally induced infections, the O titer rose at a much more rapid rate than the H titer. However, when the O titer began to drop, there was marked rise in H titer, which then remained high for

relatively long periods. For this reason, it is imperative that tests for both O and H antigens be carried out when attempting to establish a serological diagnosis of listeric infection in a suspect case.

These findings emphasized that if antisera of comparable quality are to be obtained, a rigid and consistent schedule for both antigen production and immunization must be followed if comparable results are to be obtained. It is interesting that Robbins and Griffin (218) emphasize that, for production of O antibodies, "Spontaneously occurring, smooth, non-motile variants are the material of choice."

One of the principal disadvantages of the agglutination test, and almost all other serological tests involving *L. monocytogenes*, is the marked tendency, particularly of type I O antigens, to cross-react with other bacteria. This may occur with enterococci, *Staphylococcus aureus*, and possibly some of the nonmotile corynebacteria, β-hemolytic streptococci, and coliform group (167, 223, 226, 235, 236, 280).

Precipitation reaction. Until rather recently, Drew (54) was the only one who showed an interest in precipitin reactions with *L. monocytogenes*. She found two distinct immunological groups among the 16 cultures studied, using polysaccharide extracts. However, she fell into the same error that Julianelle did (118), and classified the groups as rodent and ruminant. She even speculated that if the polysaccharides were released in the spinal fluid of patients with listeric meningitis, a precipitin test for the specific substance would lead to the ultimate source of the infection. One shudders to think of how some of the ideas expressed here will fare in 15 years.

The development of the Ouchterlony agar-gel diffusion technique stimulated several recent investigations on the precipitinogens of *L. monocytogenes*. According to Seeliger (235), the technique has several advantages over the agglutination test. The reactions are more distinct and there is less crossing with other bacteria. Unfortunately, with the commonly prepared extracts there is no method to distinguish between type 1 and type 2 cultures since both have the same O antigen formula. In spite of this fact, Muraschi and Tompkins (163) and Smith et al. have each worked out a satisfactory typing scheme based entirely on precipitinogens. The procedure has not been tried in other laboratories, but is deserving of their attention. Brubaker and Robbins (89) used the Ouchterlony technique to compare somatic and flagellar precipitonogens of the bacterium with O and H agglutinogens. Five somatic and one flagellar precipitinogens were found to be identical to Paterson's (194) O factors I, II, III, IV, and V, and H factor B, respectively. A distinctive band was common to types 1 and 2. O factor I could be separated into two distinct bands at low concentration, while factors II and IV of type 3 cultures appeared to be combined in a molecular complex. However, factor IV could be separated by treatment with 1.0% deoxycholate, which appeared to destroy factor II. They also found that specific antisera absorbed with soluble filtered antigen would no longer agglutinate whole cells of the bacterium.

Other serological tests. Independently but almost simultaneously, Seeliger (235) and Patocka (196) developed tests for the detection of complement-fixing antibodies of *L. monocytogenes*. The former used Boivin's trichloroacetic acid extracts as antigen, the latter, lysates prepared by 10 times freezing and thawing of Merthiolate-killed cultures. In spite of the difficulties and complications inherent in the CFT test, these authors felt that it is the most specific and most reliable test presently available for the detection of listeric infection.

So-called "cold agglutinins" were observed by Korn (131) in the serum of a patient with confirmed, perhaps chronic, listeric meningitis. The agglutinins rose and fell in parallel with the agglutination titers during the course of prolonged convalescence. They could also be produced in rabbits inoculated with *L. monocytogenes*; the sera of these animals had the same pattern as in the agglutination test. Korn (131) suggested that, if cold agglutinins are formed in all listeric infections, they may give rise to false-positive heterophile antibody reactions if the original Paul-Bunnell method is used. The Davidsohn modification resulted in negative heterophile antibody tests. However, Kristensen and Jessen (137) could not demonstrate cold agglutinins in a number of patients with confirmed listeric infection. Since the possible relationship between *L. monocytogenes* and infectious mononucleosis is still so controversial, it seems that the problem should attract the attention of inquiring immunologists who might resolve the problem once and for all.

Hemagglutination has not been completely ignored by the "*Listeria* chasers." Sachse and Potel (226) did considerable work with the phenomenon using chicken, sheep, or human O erythrocytes. They encountered marked crossing between group A and D streptococci, enterococci, and type I *L. monocytogenes*. This crossing may reflect the presence of Rantz antigen in *Listeria* as demonstrated recently by Neter et al. (167) using the hemagglutination test. In contrast, satisfactory results were reported by Jasinska and Wachnik (115), Burenkova (31), and Tripolitova (266). However, the latter authors

apparently made no attempt to determine specificity of the test. Further work remains before definite conclusions and accurate evaluation can be made of the value of the hemagglutination test.

More recently, Potel and Degen (212) applied the so-called "growth test" of Wellman to studies with *L. monocytogenes*. The latter consists of attempts to culture the bacterium in known dilutions of its specific antiserum in nutrient broth. As the bacterium grows in the lower dilutions, the cells agglutinate and form flocculent masses leaving a clear supernatant fluid; in higher dilutions, only a small button of growth is formed, with the broth remaining somewhat turbid. Uniform clouding of the broth with formation of a small button at the base of the tube indicates a negative result. The authors claim the test is satisfactory both as a means of serological typing with specific factor sera and as a potential diagnostic tool. A titer greater than 1:50 is regarded as specific.

In a 1954 publication, Jaeger and Myers (235) described a surface or L antigen of *L. monocytogenes* which could be absorbed by *Escherichia coli* K8 antiserum. The L antigen was lost after repeated transfer at 37 C, but was retained for 6 months or more in cultures grown at 5 C; it was thought to be related to virulence in much the same way as are Vi antigens of *Salmonella*. Since only cultures containing L antigen were pathogenic for mice, Jaeger and Myers postulated that this circumstance could account for the enhanced pathogenicity of cultures grown at 5 C. The idea is plausible in view of subsequent observations that cultures grown at 5 C are more pathogenic than those grown at 37 C (89, 146). The L antigen was destroyed by extraction with chloroform. Further extraction with ether produced milky material which reacted with *E. coli* K8 antiserum; it was precipitated by homologous antiserum and was nonpathogenic, but produced monocytosis similar to that by the monocyte-producing agent of Stanley (253). These authors promised publication on further investigations with the L antigen. The promise has not been fulfilled, nor has anyone else undertaken the study.

Serological Diagnosis of Listeric Infection

In approaching the controversial subject of serological diagnosis of listeric infection, one can find great consolation in the words of Ecclesiastes when the Preacher said, "I have seen all the works that are done under the sun . . . yea, my heart had great experience of wisdom and knowledge. And I gave my heart to know wisdom, and to know madness and folly; I perceived that this also is vexation of spirit. For in much wisdom is much grief: and he that increaseth knowledge increaseth sorrow."

Literally, legions of publications from every corner of the earth have come into the literature dealing with *L. monocytogenes*. Sera from man, almost every domestic animal and bird, and some feral species, who reside anywhere from Australia to India to Europe to the United States have been subjected to agglutination, precipitation, complement fixation, antigen fixation, hemagglutination, both direct and indirect, skin tests, growth tests, and perhaps still other tests in search for antibodies against *L. monocytogenes* as well as means to attach some significance to what was observed. Serum donors ranged from apparently healthy subjects to those showing symptoms suggestive of not only the commonly recognized forms of listeric infection but also disorders such as hepatitis, cirrhosis, and even multiple sclerosis. The human subjects included paupers, prostitutes, skilled workers, and scientists. Unfortunately, in spite of the laudable intentions of most authors, the only real contribution made by many was expansion of the bibliography. The mass of literature reveals confusing contradictions, repetitions, and dearth of illuminating information. In most instances there has been no regard for the well-established fact that *L. monocytogenes* may cross-react with a number of commonly occurring bacteria. The relatively low serological titers sometimes reported were almost certainly mere reflections of cross-reactions, since they often disappeared after absorption with staphylococcal or enterococcal cultures. Or, the reactions may have been an expression of one of *L. monocytogenes*' favorite tricks: autoagglutination. Neither was much regard given to how antigens were prepared or how cultures were selected. Since many individuals with no known exposure to *L. monocytogenes* showed titers ranging from trace to relatively high, little effort was made to determine whether such titers should be considered significant. As in the days of the early Judges of the Twelve Tribes of Israel, "Every man did that which was right in his own eyes"—consequently, reported results are not comparable.

The great controversy over the significance of serological findings in suspected listeric infections is further reflected by the 18 pages devoted to it by Seeliger (235) in his monograph *Die Listeriose*. From all the turmoil, both that imprinted firmly and bound tightly into the volumes of scientific literature and the more fleeting and elusive oral expressions, one thing is clear today, namely that diagnosis of listeric infection can be made only by isolation of *L. monocytogenes*! Some may take exception to this conclusion. But the state-

ment brooks neither controversy nor belligerence. Rather, it invites competent serologists to muster their incontrovertible evidence and data.

Agglutination test. In the middle 1950's, Seeliger and co-workers (235) found that heat-stable (O) antigens of types 1 and 3 cultures gave marked crossing with Lancefield's group D enterococci and with *S. aureus*. Although all sera containing O agglutinins against *L. monocytogenes* also contain O agglutinins against *S. aureus*, absorption with the latter has relatively little effect on type 1 agglutinins. However, it completely removes all trace of type 3 agglutinins. Cross-reactions also occur with type 4 cultures, but these are not as bothersome as those with the other serotypes. Formalin-killed cultures may also cross-react, but to a lesser degree than heat-killed cultures. Welshimer (280) recently demonstrated cross-reactions between formalin-killed *L. monocytogenes* cultures and *S. epidermidis*. When rabbits were immunized against *S. epidermidis* and challenged with *L. monocytogenes* after a 3-month lag, there was marked rise in staphylococcal antibodies. *S. epidermidis* produced similar booster effects in rabbits immunized against *L. monocytogenes*, strongly suggesting that the two bacteria share a common antigen or antigens. Since enterococci and staphylococci occur so commonly in nature, these findings greatly complicate interpretation of agglutination reactions in sera from subjects with no known exposure to *L. monocytogenes*.

Many apparently normal subjects have agglutinating antibodies against *L. monocytogenes*, but it is impossible to determine their significance. Generally, antibody in blood serum is an indication of previous exposure to a specific antigen. However, since *L. monocytogenes* may cross-react with other bacteria, the antibody may not necessarily be specific for *L. monocytogenes*, especially when titers are very low. Low response makes it difficult to establish what a "significant" titer might be. Suggestions range all the way from 1:25 to 1:800. Seeliger (235) suggested that, in the absence of clinical symptoms simulating listeric infection, a titer of 1:320 should be considered the minimal significant titer. However, this value, too, can lead to complications. Gray (89) found type 1 O titers of 1:640 in sheep maintained at the Montana Veterinary Research Laboratory. New animals have never been introduced into the flock, and listeric infection has never been known to occur in this part of Montana. In contrast, sera taken from cattle in eastern Montana several weeks after abortion of fetuses infected with type 1 cultures showed almost negligible titers against the same lots of antigen. These conflicting observations imply that the agglutination test has value only when a rise in titer can be demonstrated during the course and convalescence of disease simulating listeric infection, or if there is high titer against both O and H antigens of *L. monocytogenes*. However, this, too, can lead to complications, since usually H antibodies do not appear in high titer until after the O titer has reached or passed its peak (223, 235). For these reasons, extreme caution is required in interpreting the significance of agglutination reactions by sera from either animals or man.

The occurrence of agglutinating antibodies in sera from various domestic animals has been the subject of numerous studies in efforts to assess the possible role that animals may play in the epidemiology of listeric infection. Titers of 1:640 and higher are frequently reported, and they are usually interpreted as true reflections of experience with the bacterium. Regrettably, only a few investigators absorbed their sera with staphylococcal antigens. Gray (89) found titers of 1:640 against *L. monocytogenes* type 1 O antigen in sera from cattle in northwestern Montana and southeastern Nevada. Titers against type 4 O antigens were generally negligible. In almost every instance, absorption with *S. aureus* O antigen completely removed the activity against *L. monocytogenes*. This finding strongly emphasizes the statements of Neter et al. (167) and Seeliger (235) that all sera must be absorbed with *S. aureus* if the true titer against *L. monocytogenes* is to be ascertained. Since most investigators failed to carry out such absorptions, their results cannot be accepted as valid indicators of antibodies against *L. monocytogenes*. The unfortunate consequence is that their contributions are virtually negated.

Precipitation test. Seeliger (235) also reported cross-reactions between precipitinogens of and antibodies for *Streptococcus faecalis* and *L. monocytogenes*. The reciprocal reaction did not occur, suggesting that the precipitation test may have advantages over the agglutination test. This observation was supported by Muraschi and Tompkins (163), who found the agar-gel technique highly specific for detection of *L. monocytogenes* antibody. The latter test should be given more consideration than it has received to date.

Complement-fixation test. Seeliger (235) felt that complement fixation was the most specific of any test available, although with it, too, it is necessary to absorb sera with *S. aureus* to prevent cross-reactions. Because of technical difficulties inherent in any complement-fixation test, he recommended repeating each test a number of times or conducting it in conjunction with other tests. A titer of 1:10 is considered significant.

Presence of Rantz antigen. Rantz antigen is an antigen of undetermined chemical composition common to many gram-positive bacteria. Its presence in cultures of *L. monocytogenes* was demonstrated recently by Neter et al. (167) using the hemagglutination test. This observation casts further doubt on the validity of serological findings with this bacterium. In fact, these authors could not demonstrate antibody species-specific for *L. monocytogenes* since all *L. monocytogenes* antibody could be absorbed completely by *S. aureus* and *B. subtilis*. Neter et al. suggested absorbing sera with both these bacteria when attempting to detect antibody against *L. monocytogenes*. Their findings may also help explain the cross-reactions between streptococci and *L. monocytogenes* reported by Sachse and Potel (226) and the failure to develop a satisfactory hemagglutination test for the detection of listeric infection. Group A, B, C, and viridans streptococci all contain Rantz antigen. It is also found in enterococci, which may be the basis for the crossing between this group and *L. monocytogenes* reported by Seeliger (235, 236). This cross-reaction also shadows the reports of Burenkova (31) and Tripolitova (266) in the Soviet Union and Jasinska and Wachnik (115) in Poland on the successful use of the hemagglutination test for detection of *L. monocytogenes* antibody. It has not yet been determined whether Rantz antigen may be responsible for the failure of some investigators to produce species-specific conjugates of *L. monocytogenes* from fluorescent-antibody studies, but this possibility has been suggested by Miller (89). The sum of these findings dictates extreme caution in making a diagnosis of listeric infection based only on serological evidence; it also discredits the majority of publications reporting high listeric antibody titers in the serum from apparently normal human and animal populations.

Persistence of titer. Another disconcerting factor to confound serological diagnosis of listeric infection is the well-established fact that high titers produced either by deliberate injection of an antigen or resulting from naturally induced infection are usually fleeting. Sometimes they may persist for months, rarely years, making it risky to assign any real significance to high titers, months or even years after suspected infection, especially when paired serum samples are not available. The inconsistency of titer, coupled with unexplained high titers in some individuals with no known exposure to the bacterium and with no history simulating listeric infection, casts a long shadow of doubt on a number of reports of suspected listeric infection based only on serological evidence. Similarly, studies on the possible role of *L. monocytogenes* in mental defects of children (144) and adults (223, 251, 263), and some suggestions that the bacterium may be involved in certain cases of habitual abortion (222, 235, 236) in which the bacterium's presence was never established by culture must be regarded with suspicion. Admittedly, the circumstantial evidence is tantalizing, but science is not content with being tantalized. It demands a solid grasp. Circumstantial evidence has value only as a stepping stone to ford the swiftly flowing, fickle stream of observations beyond which lies the truth.

Serodiagnosis based on fluctuations in titer in a series of acute and convalescent serum samples has a firmly established place in modern medicine. There is no reason to question a diagnosis of listeric infection based on such fluctuations during or after a clinical course suggestive of listeric infection. However, a diagnosis made in the absence of such a course or on a single serum sample is based on a foundation no more firm than the blood clot remaining after the serum was withdrawn!

It must be admitted that many of the patients of Rost et al. (224) and Seeliger (235, 236) had high titers only at the time they aborted. However, the possibility that they were, in fact, measuring some other change in blood serum that may occur as a result of abortion was not eliminated. This idea is supported by the fact that, even in many cases of confirmed listeric infection, there is no rise in detectable antibodies (89, 136, 223, 235). It is especially true of women who give birth to infants from whom the bacterium was isolated. This situation is primarily an infection of the uterine contents with almost no involvement of the mother other than low-grade septicemia or metritis or both, neither of sufficient severity or duration to stimulate antibody production. For this reason, it seems unlikely that, in cases in which infection is so light as to preclude detection by culture, there would or could be marked rise in antibody titer. It is difficult to justify diagnosis of listeric infection, based strictly on serological evidence, in a relatively large number of patients when in no instance has such a diagnosis been confirmed by isolation of the suspected bacterium. Although Rappaport et al. (214) isolated *L. monocytogenes* from the genital tract of a number of women with histories of habitual abortion, they, unfortunately, made no mention of serological studies on their patients. Had serological studies been done, they might have found the key to untangle a mass of confusion.

If, as available information indicates (235), antibody against *L. monocytogenes* is not pro-

duced by the human subject during the first 5 to 6 months after birth, it is difficult to justify the suggestion that relatively high titers in some children with retarded mental development may reflect inapparent listeric infection during the perinatal or neonatal period. Chronic mental disorders in adults and habitual abortion cannot be satisfactorily explained until a reliable serological test is developed for the detection of previous exposure to *L. monocytogenes*.

Skin or allergic test. In an effort to overcome some difficulties of serological diagnosis, attempts have been made to develop satisfactory skin or allergic tests to detect experience with *L. monocytogenes*. Eveleth et al. (59) prepared a "listerin" using the method of Koch for the preparation of old tuberculin. Local swelling with an inflammatory reaction is the basis of a positive test. The authors claim acceptable results with sheep, but trials on only four human subjects were inconclusive. Two veterinarians with frequent exposure to listeric infection showed reactions, one accompanied by general malaise and headache of 24-hr duration. Two sheepmen with no exposure to the disease had no reaction. Dedié (47) produced an immediate reaction to skin injection of endotoxin and polysaccharide extracts in pigs, characterized by reddening of the injected area. Potel (89) also found that polysaccharide extracts gave nonspecific reactions, but claimed encouraging results with a polypeptide fraction. This incited reactions in several individuals who had relatively high agglutinating titers against *L. monocytogenes*.

Reichertz and Seeliger (214*a*) used heat-killed cells of *L. monocytogenes* as skin test antigens in patients whose agglutinin and complement-fixation titers were determined before and after the skin tests. They suggested that increases in the complement-fixation titer following positive skin tests may be of diagnostic significance in listeric infections.

Antigen-fixation test. Recently Njoku-Obi (169) obtained promising results by application of the filter-paper, antigen-fixation test of Castenada (33) to *L. monocytogenes* studies. Preliminary results with sera from sheep, cows, and man suggested that this test is more specific than presently available tests; it does not yield cross-reactions with other bacteria, and it may be capable of detecting "true" antibodies against *L. monocytogenes* even when the latter may be of low titer or after exposure to the bacterium several years earlier. One of the most promising features is that results are apparently not obscured by so-called "normal titers." Considerable work remains before a just evaluation can be made. However, if the test were applied to sera from patients with mental deficiencies or repeated abortions, a better understanding of the results obtained with the other commonly employed serological tests might be forthcoming. It has not been determined what type of antibodies the test measures, although they appear to be neither agglutinating nor complement-fixing. Whatever their nature, they may be an important stepping stone toward truly reliable serotests for detection of listeric infection. Until the goal of reliable serological or sensitivity tests is reached, it is evident that diagnosis of listeric infection can be made only by isolation of *L. monocytogenes*!

L Forms

Several investigators, among them Smith (89) and Sword (89, 260), obtained evidence that there may be a filterable form of *L. monocytogenes*. Unfortunately, their work remains unpublished. Solomkin (249) suggested, without substantial supporting evidence, that filterable forms which must be conditioned to artificial media may be responsible for the difficulty often encountered in isolating the bacterium from infected tissue. However, none of these investigators used the term "L form."

Several German and Czechoslovakian microbiologists postulated that a filterable or L form actually crosses the placenta in perinatal listeric infection, since the placenta is refractory in invasion by most bacterial cells. The proposal is questionable, since a preponderance of evidence indicates that *L. monocytogenes* can very quickly penetrate the placental barrier (84, 95, 159, 198, 223, 234, 235). Nevertheless, Suchanova et al. (259) initiated a series of studies by implanting collodion sacs with a pore size smaller than *L. monocytogenes* cells into the peritoneal cavities of pregnant and nonpregnant rabbits. In eight of 11 rabbits, the bacterium penetrated the placenta without development of "significant" bacteremia. Although the authors did not positively state it, they strongly implied that there was penetration by minute or filterable forms. However, the implication may not be capable of proof. Since the very first report by Murray et al. (164) numerous authors have reported difficulty in isolating the bacterium from the blood of either artificially or naturally infected animals. Within the limits of study reported (259), bacteremia could have passed undetected. The presence of small granular forms, approximately the size of *Rickettsia burneti*, located within histiocytes was offered as further support for the development of L forms. The granular forms developed into typical *L. monocytogenes* when cultured on artificial media. The study was not

particularly convincing of the presence of either L or filterable forms of *L. monocytogenes*.

In a simultaneous but more convincing study, Suchanova and Patocka (258) produced L forms by cultivating the bacterium on media containing either penicillin or glycine. Growth in the presence of penicillin produced minute colonies composed of small cocci, glycine produced long filaments and some large spheroid forms resembling L forms, and, when the two substances were combined, the cultures appeared identical to Dienes' type B L forms. Such cultures stimulated antibody against *L. monocytogenes*; they regained their normal form after several subcultures. Intracellular minute coccoid or granular forms were seen after injection of the bacterium into rabbit muscle together with a Freund-type, lipid adjuvant; development of similar forms in subcutaneous tissue or in chicken embryo yolk sacs followed implantation of collodion sacs and was given as further evidence of the existence of L forms. These few reports make it apparent that a great deal more work remains before the question of L forms in cultures of *L. monocytogenes* is satisfactorily answered.

Phage

A number of investigators have confided intention to dabble with the phages of *L. monocytogenes*. Although the presence of phages in cultures of *L. monocytogenes* was mentioned by Schultz (233) as early as 1945, Sword and Pickett (260), Guillot and McCleskey (94*a*), and Jasinska (114A) have moved beyond this stage of intent and shared their findings with the general scientific community. Although the former (260) succeeded in demonstrating the presence of phage by the cross-streaking and spotting method, this procedure was generally inadequate since the bacterium's growth is normally thin and transparent, making plaque detection difficult. This difficulty led them to attempt ultraviolet induction to stimulate maturation of provirus. A 60-sec exposure of a tryptose broth culture in an open petri dish to ultraviolet rays of 2,537 A followed by incubation at 37 C for 90 min produced more than a 3-log increase in phage titer. Increasing the irradiation time had no beneficial or detrimental effect on either phage or culture. The principal disadvantage of the method was production of substances similar to colicins which led to many false-positive plaques. A total of 18 phages was isolated from 121 cultures. These phages could be divided into two somewhat similar groups. However, five were sufficiently different that they could be used for typing purposes. Phage-typing revealed groups corresponding almost exactly to the serotypes of Paterson (194). There was no evidence of cross-reaction with other bacteria as commonly occurs with the usual serological tests (167, 223, 226, 235, 236, 280). As with serotypes, phage groups had no relationship to either host species or geographic origin of the cultures. These authors feel that phages possess potent potential both for identification of suspect cultures and for typing purposes in epidemiological studies.

Morphologically, all phages were similar if not identical when examined with an electron microscope. The head measured 85 to 90 mμ and the tail 265 by 15 mμ. All phages studied were temperate, but variants were found which appeared to be virulent.

It may be of more than passing interest that type 3 cultures appeared to be phage-resistant. However, because of the rarity of this serotype, it would be difficult to accumulate sufficient cultures for work to permit valid conclusions. Type 3 is known to react distinctively in the agglutination and complement-fixation tests (235); it differs chemically from the other serotypes (268). These findings suggest that the type is perhaps distinctive in many respects and is worthy of more attention than has been given to it.

Recently, Watson and Eveland (276*a*) and (276*b*) described the isolation of a phage, designated L11/16, which lysed 100% of *L. monocytogenes* serotypes 1, 2, and 4 and 50% of type 3. An anti-L11/16 phage serum was prepared in rabbits and conjugated with fluorescein isothiocyanate. A procedure was developed for applying phage-fluorescent antiphage staining to the specific identification of *L. monocytogenes* in culture and in sections of infected mouse tissue. The L11/16 phage was added to the culture or tissue section to allow attachment of the phage particles to the surface of the bacterium. After washing to remove the unadsorbed phage, anti-L11/16 phage FA was allowed to react with the adsorbed phage on the surface of the bacterium. When viewed in a fluorescence microscope, the staining pattern was distinct from ordinary methods of fluorescent staining by antibacterial serum, in that organisms appeared irregular and often bizarre. The absence of staining of heterologous bacteria with this method suggests that phage-fluorescent antiphage staining may be a useful adjunct method in the identification of *L. monocytogenes*.

Hamon and Peron (95*a*) described the production of antagonistic substances showing the general properties of bacteriocins after ultraviolet irradiation of cultures of *L. monocytogenes*.

Chemical Composition

Comparatively few studies have been directed toward determining the chemical composition of *L. monocytogenes* cells. Roots (222) obtained cell walls of the bacterium by ether extraction followed by 4 hr of agitation with glass beads and enzymatic digestion. He found marked differences in electron density as a result of treatment of the wall with ribonuclease, trypsin, and deoxyribonuclease, suggesting that the cell wall contains relatively large quantities of substrate for these enzymes.

Keeler and Gray (125) could not disrupt the bacterium by either direct osmotic lysis or various modifications of Weibull's lysozyme method (278) for protoplast formation of gram-positive bacteria. However, they obtained satisfactory disruption by sonic oscillation (10 kc at 1.1 to 1.2 amp at 4 C for 16 min) and recovered the cell wall by differential centrifugation. Wall material collected in this manner contained about 20% hexose, consisting of equal amounts of glucose and galactose, and of 5% hexosamine. About half of the wall material was Kjeldahl protein composed of five amino acids: alanine, glutamic acid, α,ϵ-diaminopimelic acid, aspartic acid, and leucine. The protoplasmic material contained from 17 to 20 detectable amino acids. The remainder of the wall could not be accounted for quantitatively. In addition, the wall contained at least 5% of the phosphorus (P^{32}), 15% of the iron (Fe^{59}), and 36% of the calcium (Ca^{45}) content of the entire cell. It is possible that the latter values could be doubled since only 30 to 50% of the wall was actually recovered by the method employed. The iron appeared to originate in the cell wall, while the phosphorus originated in both wall and protoplasmic fractions. Keeler and Gray also found that *L. monocytogenes* contained a very low content of cytochrome. Since a relatively large proportion of the iron of bacterial cells appears to be cytochrome-bound, the results with Fe^{59} suggested that some of the cytoplasmic membrane adhered to the wall of cells disrupted by sonic oscillation. Ultraviolet spectra failed to reveal the presence of nucleic acid in the wall, although it was present in relatively large amounts in the protoplasm.

The study of Keeler and Gray (125) was carried out on a single type 4b culture. Ideally, the studies should be extended to other serotypes, especially since McBride and Girard (156) showed that the various serotypes had marked variations in physiological activity that might possibly be reflected in differences in chemical composition.

Tubylewicz (268) investigated a number of different protein fractions obtained from whole cells of eight cultures of *L. monocytogenes*; these cultures represented all serotypes and had both quantitative and qualitative differences in cellular protein. Since the study was carried out on whole cells, the results cannot be compared with those of Keeler and Gray (125). The protein percentage ranged from 21.8 to 53.5 among the various strains. Four strains yielded four protein fractions; one yielded three; two gave two; and the single type 3 culture yielded only one fraction. Unfortunately, there was no obvious correlation between number of protein fractions and serotype or, if so, it was not clearly expressed. All fractions contained the same 14 amino acids, and less than 1.0% glucosamine. There seemed to be little variation in deoxyribonucleic acid content, but the cultures could be divided into two groups on the basis of ribose content, which varied from less than 1.0% to more than 13%. The one type 3 culture studied appeared to be distinctly different from the other serotypes. Whether this difference is related to this serotype's somewhat distinctive serological behavior (235) remains to be determined; it may be, since type 3 cultures also appear to be phage-resistant (260).

A number of other investigators fractionated *L. monocytogenes* in attempts to isolate pathogenicity-enhancing factors (195, 242, 253, 269). The chemical make-up of these substances remains undetermined. More than a decade ago, Stanley (253) isolated a lipid, apparently located in the cell wall or membrane (125), that incited monocyte production in monogastric animals, as will be indicated in the next section.

MONOCYTE-PRODUCING PROPERTIES

Histological Studies on Origin and Development of Monocytes

Almost immediately after the publication by Murray et al. (164) reporting the presence of large numbers of monocytes in the circulating blood of rabbits exposed to the then new bacterium, *L. monocytogenes*, histologists realized the potential value of this observation for studies on the origin and development of the monocyte. Many other substances were available, but none produced monocytes at such a spectacular rate—sometimes 6,000 times normal and without appreciable increase in the number of other leukocytes. It is regrettable that, in the many recent publications and discussions dealing with the bacterium, most of the interesting early papers by Witts and Webb (284), Bloom (203), Lang (143), Nyfeldt (223), Bianchi (19), Rezzesi (216), Wallbach (276), Penati and Levi (199), and Con-

way (37, 38) on the use of this tool in studies on the origin of the monocyte are completely forgotten. Yet, the reports present one of the most exciting and challenging mazes that any adventurous bacteriologist or histologist could ever hope to explore.

Although all of the investigators used essentially the same procedure, the intravenous inoculation of rabbits, they arrived at entirely different conclusions or none at all (143). Some believed that the monocyte was a distinct cell type with its own stem cell (19, 199, 216, 276, 284), and others believed that it was a transformed lymphocyte (25, 37). Although there is still controversy, the latter view is the one generally held. Conway (37) felt that the other histologists erred by permitting their animals to develop first monocytosis of the circulating blood and killed them much too late to comprehend the true sequence of events. She killed her rabbits and guinea pigs beginning as early as 1 hr after exposure. Within the first 48 hr, large numbers of monocytes were produced through individual transformation of lymphocytes in the cortex of the mesenteric lymph node and periarterial lymphatic tissue of the spleen. In the early stages, the lymphocytes migrated from areas of lymphatic tissue which then rapidly produced new lymphocytes; many mitotic figures were seen in the lymphatic tissues.

Both Bloom (25) and Conway (37) had no difficulty tracing the various transitional forms from lymphocyte to monocyte. The actual transformation took place within the blood vessels all over the body, particularly in sinuses of the spleen and liver, and reached its greatest extent in organs where blood flow velocity was relatively sluggish, perhaps in the capillaries of the lung. It could be interjected here that Murray (165) considered his original observation on accumulation of monocytes in the small blood vessels of tissue sections from their animals as "The Glass Slipper" in the Story of Listeria. Bloom (25) found also that splenectomized rabbits developed monocytes from the bone marrow and mesenteric lymph nodes.

In rabbits sacrificed at 60 hr, at which hour monocytosis reached its peak in the circulating blood, and at which time most investigators examined their animals, the proliferation of reticular cells might give the impression that the monocyte originated from reticuloendothelial cells. According to Conway (37), such proliferation was seen only after exhaustive lymphocytopoietic activity. When the fixed lymphocytes were completely depleted, lymphocytes developed from fixed reticular cells and mitotic figures were seen in these areas. This might give the illusion of reticuloendothelial hyperplasia, as reported by some investigators. However, even at this time the reticular cells developed directly into lymphocytes rather than into monocytes. In the early stages, many mitoses were seen in the lymphocytes but not in reticular cells.

The single thing on which most investigators agreed was that the mesenteric lymph nodes did not participate in monocyte production. Conway (37) found them to be one of the most active centers, but only during the first few hours after exposure. In later stages, it became hypoplastic and completely inactive.

In a follow-up study by Conway (38) on rabbits immunized by inoculations of living *L. monocytogenes* culture, the monocytosis progressed even more rapidly than in nonexposed animals. Also, the macrophages that developed from the monocytes ingested the bacterial cells at a greatly accelerated rate.

In occasional reports on the production of monocytes, an abnormally high basophile count is more or less casually mentioned (25, 38, 89, 235). Some have cautiously commented that these may actually be polymorphonuclear leukocytes with a large number of ingested bacterial cells. Others await an explanation. The latter may not be easy, since the leukocytosis is accounted for almost entirely by the increase in monocytes.

There is no explanation for the stimulation of monocyte production by *L. monocytogenes*. That it is not entirely a characteristic of the bacterium is suggested, since the host also participates in the phenomenon. It is a striking fact, but one almost completely neglected except for an occasional comment, that the monocytosis is produced only in monogastric animals, not in ruminants. Experimentally, Olson et al. (179) could not produce monocytosis in sheep following several different methods of exposure. Instead, there was increase in neutrophiles with decrease in lymphocytes, which is the usual picture in naturally infected ruminants (80, 85, 179, 235). It may be within the realm of possibility that there is a basic difference between ruminants and monogastric animals—a difference which dictates that in ruminants listeric infection is usually manifested by localized encephalitis, whereas in monogastric animals, and in ruminants before the rumen is functional, septicemia with or without associated meningoencephalitis is seen most often (80, 85). Septicemia is rare in adult ruminants (85), and unfortunately no reliable information is available on the blood picture in septicemic animals. It is difficult to understand why this fascinating, apparent difference and potentially rewarding subject has received so little attention.

Isolation of Monocyte-Producing Agent

A monocyte-producing agent (MPA) was found by Stanley (253) to be contained in nonantigenic, nontoxic, chloroform-soluble lipid extracted from the bacterial cell. The same lipid could be extracted from the liver of infected rabbits but not from that of normal rabbits. Both extracts stimulated monocytosis when inoculated intravenously in rabbits. However, the response to that from rabbits was not marked or of as long duration as that produced by the bacterium itself.

Gray (89) filtered broth cultures of *L. monocytogenes* through a Millipore filter. The resulting sterile filtrates inconsistently produced monocytosis of 10 to 15% when inoculated intravenously into rabbits, indicating that under certain still undetermined conditions MPA may be released in fluid medium. This observation suggested that MPA may be similar to an exotoxin in nature and that it may be released from the bacterial cell in the animal body. Such a situation may help to explain why MPA can be extracted from the liver of infected rabbits. This is another area where additional research is required.

Murray (165), in a rather serendipitous way, discovered that MPA acted as a weak Wasserman antigen and prepared an antigenic lipid protein by precipitating MPA with syphilis antiserum. Some animals immunized with the complex developed monocytopenia during immunization. There was no adequate explanation for this discovery, and the study was not extended. However, Murray (165) felt that it confirmed the specificity of monocytosis due to *L. monocytogenes*.

Unfortunately, no attempt has been made to determine the effect of MPA on ruminants or to isolate it from the liver of intravenously exposed ruminants. Neither have histologists availed themselves of this new tool for further studies into the origin, development, and function of the monocyte. It is more than likely that the picture of monocytopoiesis would be considerably altered in an environment free from the toxic and necrotizing effects of the living bacterium. Also, the histological features of the hemopoietic centers under the influence of sustained monocytosis made possible by MPA might result in a valuable contribution to the growing body of academic knowledge.

Uher and Uher (269) made a beginning on this problem when they simultaneously injected rabbits intravenously with MPA and intramuscularly with the killed bacterial suspension remaining after the chloroform extraction of the culture. Injections were continued for 8 weeks, resulting in only a 15% monocytosis at 8 weeks. These authors were misled to the same false conclusion as other investigators who retained their animals for relatively long periods. At necropsy, most rabbits had few alterations: four had marked enlargement and proliferation of the reticular cells in the liver and lymph nodes, which they called "reaction reticulohistiocytosis." They concluded that the reticuloendothelial system was the site of lymphocyte and monocyte formation. They also suggested "*L. lymphomonocytogenes*" as a new name for the bacterium since it produced large numbers of lymphocytes as well as monocytes.

In studies on the chemical composition of *L. monocytogenes*, Keeler and Gray (125) obtained evidence that MPA was located in the cell wall. Mice inoculated intravenously with cell wall fractions obtained by sonic disintegration and fractional centrifugation developed monocytosis of 17 to 24%, while those receiving protoplasmic fractions seldom showed more than 4 to 17%.

Antibody Production Under Sustained Monocytosis

Stanley (254) very quickly applied MPA to studies on antibody production. Rabbits inoculated simultaneously with *Salmonella typhimurium* antigen and MPA produced antibody more rapidly and at a greater rate than rabbits given antigen alone. Soon afterward, Girard and Murray (74) using *S. typhosa*, staphylococcus toxid, and horse serum as antigens confirmed that antibody production was enhanced four to eight times in rabbits with a sustained monocytosis due to prolonged injection of MPA. Although more than 7 years have passed since this work was published, years in which there was much interest in the so-called "adjuvants" such as mineral oil and some synthetic compounds to augment production of immunity, the promise shown by the products of this Cinderella bacterium lie neglected among the dust of the bacteriologist's and immunologist's hearthstone.

Girard and Murray (74), in an extension of these studies, produced large quantities of monocytes in the pleural cavity of rabbits with sustained monocytosis by intrapleural injections of gum arabic-beef extract mixtures. When these animals were exposed to any of several different antigens, the monocytes in the pleural cavity contained a much greater concentration of antibody than did the fluid of the exudate or the circulating blood. In an effort to determine whether antibody was actually produced in the monocyte, they found that monocytes in passively immunized rabbits contained a higher titer of passive antibody than the circulating blood. They felt

that, although the monocyte may not produce antibody, it played an important role in antibody transport. They also suggested that the monocyte may be responsible for the rapid disappearance from the blood of passively introduced antibody, and raised the question of whether antibody so stored might still be available for use, or destroyed in the cell. In a reflective mood, Murray (165) remarked, "I hardly dare speculate on the possibilities of influencing methods of active immunization, but in dreamy moments I see attractive hazy vistas only to be brought back to the realities limiting opportunity for experimental research." Perhaps someday, somewhere, someone with the opportunity may be induced or enticed to explore the hazy vistas. Another "Glass Slipper" might be waiting there.

Phagocytosis

Since we have now descended to the level of the tissue cell, we may as well take the opportunity to pry into another neglected subject—phagocytosis. As one would expect, the first work with *L. monocytogenes* was done by Murray et al. (164). They found that polymorphonuclear leukocytes from both normal rabbits and those with an induced monocytosis phagocytize large numbers of *L. monocytogenes*. The monocytes from normal rabbits had little activity, whereas those from rabbits with a monocytosis had activity equal to the polymorphonuclears. Yashenkina (286), using conventional methods, learned that polymorphonuclear leukocytes from hyperimmunized rabbits, mice, and guinea pigs were phagocytic, ranging from approximately 10% at 5 days after the last immunization to 20 to 58% after 30 days. There was no reaction in blood from hyperimmunized sheep or swine, indicating that such examination would have no value for determining the presence of listeric infection in these species of animals.

Recently, a number of studies on the effects of *L. monocytogenes* at the cellular level have been done. By working with normal and immunized mice, as well as monolayer tissue cultures of macrophages obtained from these animals, Mackaness and his co-workers (127*a*, 151*a*, 151*b*, 157*a*) studied the cellular resistance of mice to *L. monocytogenes* by examining the capacity of the organism to surive and multiply in host macrophages. During the first 3 days of primary infection, bacterial populations of the spleen and liver increased at a constant rate. On the 4th day, the host became hypersensitive and bacterial growth ceased, with rapid inactivation of the organisms. Acquired resistance to *L. monocytogenes* was associated with the development of delayed hypersensitivity to listeric antigens and with the appearance of abnormally high antibacterial activity in the mononuclear phagocytes of infected mice. Passive transfer of this resistance to nonimmunized mice could be accomplished by the macrophages, but not with the serum, of immunized donor mice. The cellular resistance to listeric infection lasted about 3 weeks. Thereafter, the mice remained hypersensitive but were unable to inactivate a challenge inoculum of *L. monocytogenes*. There was, however, an accelerated response to reinfection. This accelerated response may depend upon an ablity to generate a new population of resistant cells from a residium of specifically sensitized macrophages or macrophage precursors still surviving in the tissues. North and Mackaness (171*b*, 171*c*) conducted electron-microscopic observations on the fine structure of peritoneal macrophages of normal mice and mice immunized with *L. monocytogenes*. Fauve (59*a*) confirmed that macrophages from mice immunized with living *L. monocytogenes* were more resistant to infection with the bacterium in vitro than were macrophages from normal mice.

Njoku-Obi and Osebold (169*b*) obtained suppression of the growth of *L. monocytogenes* in tissue cultures of the peritoneal exudate cells of sheep immunized with living cultures of the bacterium. Armstrong and Sword (6*a*) obtained similar results using guinea pig monocytes. The resistance to the bacterium was due to factors within the immune cells; immune serum played only a minor role in the results. Increased cellular resistance could not be produced by injecting the donor animals with killed cultures of *L. monocytogenes*.

Sword (259*a*) studied the protein alterations induced by *L. monocytogenes* infections in mice. During the acute phase, 2 to 5 days postinfection, there was a marked increase in the α 2 and β globulins. Chronically infected animals responded with an increase in γ-globulin.

METHODS FOR ISOLATION FROM INFECTED MATERIAL

Refrigeration Method

While *L. monocytogenes* usually grows well on most of the commonly employed bacterial media after initial isolation, there is considerable evidence with both naturally and artificially induced infections that initial attempts may not always be successful (78, 80, 84, 88, 121, 157, 170, 186, 187, 188, 223, 235, 237, 248, 249, 277). Murray et al. (164) called attention to this problem in their original report in 1926; so did Gill (71) and most other early investigators, yet the majority of con-

temporary bacteriologists have consistently ignored it. Acknowledgment of the situation by Rappaport et al. (214) may have contributed to their recent success in isolating the bacterium from patients with histories of habitual abortion.

The various techniques which have proved most successful for isolation of *L. monocytogenes* generally stress the need for tissue maceration. The bacteria are often incarcerated in the focal lesions of infected tissue, frequently within the cells. The method that has proved most effective consists of macerating suspected tissue in a mortar or Waring Blendor together with a few milliliters of sterile distilled water or nutreint broth. Saline should be avoided since it may harm the bacterium, especially if the population is low. A portion of the suspension is plated on blood-agar, tryptose-agar, Trypticase Soy Agar, Eugonagar, or modified McBride's agar (15, 156), and the remainder is stored at 4 C. Body fluids, swabs, etc., are similarly plated, and a portion is stored at 4 C. The plates are incubated at 37 C for 18 to 24 hr and examined with a scanning microscope, or a hand lens with the plate resting on a laboratory tripod (15) and with obliquely transmitted illumination as described by Henry (102). When viewed in this manner, 18- to 24-hr-old smooth colonies of *L. monocytogenes* are distinctively blue-green and so characteristic that with a little practice they can be identified quickly even in highly contaminated cultures (78, 82, 89, 97), or distinguished from a number of other common pathogens (98, 152). When the population of contaminants is high and that of *L. monocytogenes* is low, small sectored colonies, if present, can easily be recognized. In spite of the black color of colonies on potassium tellurite plates, *L. monocytogenes* can be recognized by the blue-green margin of the colony (79, 82, 97, 98). Although colonies which develop on blood-agar or other colored media possess essentially the same morphological characteristics, they lack the distinctive blue-green color and cannot be readily distinguished from other pathogens or contaminants. Rough colonies which may differ considerably in morphology or color from smooth colonies are seldom encountered in isolations from infected tissue (83).

If the initial culture fails to reveal *L. monocytogenes*, the tissue suspension, fluids, swabs, etc., which were refrigerated, should be replated at intervals of several days for a period of at least 3 months. Usually only a few days or a few weeks of refrigeration are required for the bacterium to appear, but in a number of instances 30 to 50 days elapsed before the bacterium was isolated (78, 80, 84, 121, 186, 237, 277). In one instance, Kampelmacher (121) found it necessary to refrigerate calf brain material 6 months before the bacterium could be detected. Admittedly, this method is slow, has serious disadvantages for the diagnostician, and is cumbersome for the laboratory staff; yet it has been shown repeatedly to greatly enhance the probability of isolating *L. monocytogenes*. Osebold and Inouye (186) observed an increase of 47 to 95% in the number of isolations from artificially infected rabbits and sheep; more recently, Osebold et al. (188) reported an 11% increase in the number of cultures from artificially infected bovine tissues and a 60% increase from naturally infected bovine tissues, while Seeliger and Plab (237) reported a 23% increase in tissues from mice with artificially produced chronic listeric infections. These findings emphasize that the possibility of listeric infection cannot be eliminated merely by failure to isolate the bacterium by initial attempt at culturing it.

The mechanism of the enhancing effect at 4 C is not understood. When it was first described by Gray et al. (78) in 1948, it was suggested that it might involve an inhibitory factor in bovine brain. Attempts to demonstrate such a factor in brains from several different species have been unsuccessful (89, 162). During the intervening years, it has been shown repeatedly that delayed growth of listeria from infected tissue is not limited to bovine brain, but that it prevails in all animal and human tissue and body fluids. Enhancement by cold storage has been used successfully to isolate the bacterium from such widely diversified sources as pneumonic lungs of infants (157), vaginal swabs from women (87), lemming brains (170), and silage extracts (88).

Since it is well established that *L. monocytogenes* will grow slowly at 4 C, mere multiplication may play some part in the phenomenon. However, the increase in colony numbers is so rapid at times that it seems unlikely that enhanced isolation is due entirely to mere multiplication. There are strong indications that other still obscure factors, perhaps chemical or enzymatic, also play a part. Solomkin (249) suggested that it may be related to a filterable form of the bacterium which must be adapted to growth on artificial media. In support of these ideas, Harbour and Gray et al. (85) each reported large numbers of bacteria resembling *L. monocytogenes* in intestinal smears from calves that died of listeric septicemia, but from which they could not isolate the bacterium. Csontos et al. (42) reported that some cultures prepared from organs of naturally infected geese failed to yield bacterial growth, although stained impression smears from the same organs revealed large numbers of bacteria

resembling *L. monocytogenes*. More recently, Wedemeyer and Seeliger (277) isolated the bacterium from meconium of an infected infant only after the meconium had been refrigerated for 5 weeks; in this instance, the bacterium could be seen clearly in Gram-stained smears of the meconium. These authors stated that several other laboratories in Germany experienced similar difficulties while attempting to isolate the bacterium from meconium.

The study by Smith et al. (248) suggested that delay in growth might be related to the bacterium itself. When rabbit spleen cultures were infected with *L. monocytogenes*, the bacterium could be recultured easily from the preparation when it had been in contact with the tissue for 24 hr or less. However, after 48-hr contact, the bacterium often failed to grow on artificial media, even though the characteristic tumbling motility and production of lesions could be seen by phase-contrast microscopy. When these same tissue culture preparations were refrigerated for 4 days or more and recultured in the same way, the bacterium grew out in large numbers. Recently, Girard (89) encountered essentially identical results with human stools inoculated with *L. monocytogenes*. The bacterium could be isolated only after the stool had been refrigerated. Whatever the cause of the delayed growth may be, it emphasizes the need for more effective methods of isolation to avoid possible delays in confirming clinical diagnoses of listeric infection, and to prevent erroneous diagnoses when initial cultures fail to reveal the bacterium.

Other Methods

Many methods have been suggested to coax *L. monocytogenes* to propagate on artificial media. Olson et al. (180) compared a number of methods including duplicate culturing from each organ, inoculation of mice with suspensions of suspected tissue, storage of ground tissue in glycerine, centrifugation, and use of selective media to isolate the bacterium from tissues of both naturally and artificially infected sheep. Best results were obtained by grinding duplicate samples of fresh tissue and inoculating them into tryptose broth. With this technique, Olson et al. (180) were later successful in isolating *L. monocytogenes* from 94% of the sheep brains cultured. Unfortunately, they did not compare this procedure with the refrigeration method described in the preceding paragraphs. The latter gave 100% isolations from more than 150 sheep and cow brains which had perivascular cuffing and focal necrosis characteristic of listeric encephalitis (80, 89).

Perhaps as many different media have been recommended as there are individuals who have made serious efforts to isolate *L. monocytogenes*. Zink et al. (287) favored culturing pieces of tissue in thioglycolate broth; Kemenes (126) preferred horse meat broth with 0.3% sodium thioglycolate and 10% sheep blood; Simon (243) recommended preliminary culture in either brain-heart-dextrose broth, placenta broth, or thioglycolate-serum medium; and more recently Rappaport et al. (214) used a medium composed of tryptose (Difco), beef extract, sodium chloride, dextrose, agar, and vitamin B_1 to isolate *L. monocytogenes* from the genital tract of women with histories of habitual abortion. The list could be extended almost indefinitely, but would serve little real purpose other than to add more names and more media—and maybe contribute confusion. The commonly employed laboratory media presently available are quite satisfactory, providing the bacteriologist is aware of the difficulties that may be encountered in isolation attempts. Gray (82) recommended Tryptose Agar (Difco), with or without blood, and tryptose broth. Many others also swear by this medium; perhaps an almost equal number swear at it.

Selective Media and Methods

Several efforts have been made to develop satisfactory selective media for the isolation of *L. monocytogenes*. Gray et al. (79) suggested inoculating contaminated material into nutrient broth containing 0.05% potassium tellurite, incubating at 37 C for 6 to 8 hr, and then plating on either plain Tryptose Agar or Tryptose Agar with 0.05% potassium tellurite. The procedure appeared to give very satisfactory results. However, shortly after publication of their paper, the authors learned, as have many others so naive as to publish a selective medium for any bacterium, that it did not always work. Olson et al. (180) observed inhibition of many strains. This finding has been confirmed in several laboratories and, hence, the utility of the procedure is limited considerably. It is possible, also, that variation in growth on potassium tellurite medium is a reflection of differences related to serological type. Recently, McBride and Girard (156) attempted to develop a selective method for the isolation of *L. monocytogenes* but found physiological differences among the four serotypes, which might interfere not only with their ability to grow on selective medium, but also impair isolation from infected material.

The selective method developed by McBride and Girard (156) was based in part on the report of Shimizu et al. (240), who reported that

1:10,000 concentration of guanofuracin (5-nitro, 2-furfurylidene aminoguanidine hydrochloride) in culture medium suppressed many groups of bacteria without interfering with growth of *L. monocytogenes*. The method recommended by McBride and Girard employs enrichment broth composed of Tryptose Phosphate Broth (Difco) to which is added Furacin (nitrofurazone) to make final concentration of 1:100,000. The selective medium is composed of phenyl ethanol agar base (Difco) with 0.05% lithium chloride, 1.0% glycine, and 5.0% added blood. The material to be cultured is inoculated in the enrichment broth and incubated at 37 C for 48 hr. It is then plated on the selective medium and incubated at 37 C for an additional 24 to 48 hr. Colonies of *L. monocytogenes* are gray in color and produce no hemolysis. This procedure proved very satisfactory for isolating *L. monocytogenes* from artificial mixtures of a variety of commonly encountered pathogens and contaminants. However, it is too early to evaluate properly its effectiveness under clinical laboratory conditions.

Bearns and Girard (15) found that the selective medium described above also supported good growth of *L. monocytogenes* without the addition of blood (modified McBride's medium). The plates could be examined by use of Henry illumination (102), and colonies of *L. monocytogenes* could be detected easily by their characteristic blue-green color (82, 83).

Kampelmacher (121, 223) added 0.05% potassium tellurite and 0.2 mg of chloramphenicol per 100 ml to meat extract-agar, and found this to be a very satisfactory medium for isolations of *L. monocytogenes*. However, Seeliger (235) observed only poor growth among some 300 stock cultures placed on this medium. He raised the interesting question of why stock cultures should grow poorly on it while freshly isolated cultures apparently grew very well. One may speculate that there is a relation to the failure of some cultures to grow initially on artificial media.

Although not all cultures of *L. monocytogenes* grow on media containing potassium tellurite, it is used rather extensively in many laboratories. It is noteworthy that, although the colonies on colorless media are black, they still retain a characteristic blue-green at the margin when viewed by Henry illumination (79, 82, 98). Micrococci and streptococci also grow on this medium. However, micrococci have an intensely black center and pinkish-yellow periphery, while streptococci are smaller and pinkish-grey, with a dull surface (79, 98, 152).

Since *L. monocytogenes* can tolerate relatively high concentrations of salt, their use has been suggested as a means to isolate the bacterium from contaminated material (255). Simon (243) reported good growth at concentrations as high as 10%. However, few investigators could confirm this finding. Flamm (63) claimed that the bacterium did not survive in 2.5% salt, and Gray (89) found that even physiological saline could kill the bacterium, especially if the population was very small. For this reason, saline should be avoided when preparing tissue suspensions for culture.

Very recently, Robin and Magard (217) recommended culturing at 45 C on Hajna-Perry medium or at 47 C in liquid Rochiax medium to differentiate *L. monocytogenes* from *E. insidiosa*, several common streptococci, and diphtheroids. They also reported satisfactory selective results with Brilliant Green Agar containing bile. These media should prove useful since *L. monocytogenes* grows well at the suggested elevated temperatures.

During study of artificially induced listeric infection in the genital tract of female rabbits, Gray et al. (82, 84) made use of the bacterium's ability to grow almost as well at room temperature as at 37 C. Vaginal swabs and exudate, fetal fragments, etc., were plated directly on Tryptose Agar. Incubation at ambient temperature effectively inhibited most contaminants, particularly spreaders, without interfering with growth of *L. monocytogenes*. The method has considerable merit and should not be shunned by well-educated bacteriologists merely because it is so simple.

Before leaving the subject of selective media, it might be appropriate to mention the effect of various dyes on *L. monocytogenes*. Actually, this matter has received surprisingly little attention. Pacheco and Santos (191) tested nine strains of *L. monocytogenes* representing all serotypes against 31 different dyes. Almost all dyes tested exerted some inhibitory effect. The most effective were nigrosin, methyl violet, crystal violet, methyl green, Sudan III, basic fuchsin, and water-soluble erosin.

Ryu (225) reported that citronella oil has very little bacteriostatic effect on *L. monocytogenes*.

Since *L. monocytogenes* may often be encountered in mixed bacterial populations, especially in cultures made from the genital tract, and since it may be so easily confused with some hemolytic streptococci or nonpathogenic diphtheroids, a means of readily identifying the colonies would be most desirable. Kröger (138) made use of the so-called "saccharose indicator plate" containing water-soluble aniline blue as indicator. Colonies of *L. monocytogenes* de-

veloped blue color while most other bacteria did not develop blue color or developed it less rapidly than *L. monocytogenes*. Seeliger (236*a*) felt that β hemolysis which develops on blood plates is fairly characteristic, and developed a cultural isolation scheme based on sheep blood-agar, motility testing, and agglutination of the culture with specific antisera. Not all cultures are hemolytic.

Most investigators who attempted the Henry lighting technique (102) as suggested by Gray et al. (82) found it to be one of the most satisfactory methods for rapid identification of colonies of *L. monocytogenes*. Its value was emphasized in a series of investigations by Gray et al. (84) on artificially induced perinatal listeric infections in which it was necessary to demonstrate the bacterium's presence in such grossly contaminated material as urine, vaginal exudates and swab cultures, drinking water, etc. Hartwigk (97), working with artificially infected milk samples detected colonies of *L. monocytogenes* without difficulty even when they were outnumbered by other bacteria 50,000 or more to 1. It is doubtful that the presence of *L. monocytogenes* could have been detected in these specimens without this technique. Recently, Malakhov (152) reported that, by this method, colonies of *L. monocytogenes* could be distinguished from those of 25 other bacteria commonly encountered in diagnostic laboratory work. The group included members of the coliform group, *Salmonella* sp., *Pasteurella* sp., and various streptococci and staphylococci. The principal disadvantages of the procedure are that most diagnostic laboratories do not routinely use colorless media for isolation from infected material, and that the laboratory staff may not have had prior opportunity to view colonies of the bacterium.

Differentiation from Erysipelothrix insidiosa

For some poorly explained reason, there is persistent notion that cultures of *L. monocytogenes* and *E. insidiosa* are virtually indistinguishable from each other. Since I have the dubious distinction of making the first recorded isolation of both these bacteria from animals in Michigan, it is difficult for me to comprehend this. Nevertheless, the isolations have furnished material for numerous publications, most of which further emphasize the difference without presenting convincing evidence of similarity. Very early, Barber (13) compared various characteristics of these two bacteria and concluded that, except for the superficial fact that both were gram-positive rods, there was little similarity. Others soon reached the same conclusion that there are distinct differences both in colonial and cellular morphology sufficiently characteristic to be distinguished without the aid of elaborate schemes, diagrams, selective media, and color reactions.

According to Ilina (110), *L. monocytogenes* discolored meat peptone broth containing methylene blue and neutral red in 4 hr, whereas *E. insidiosa* had no effect on it. Plashke (203) confirmed this observation, but found that 18 hr was required to produce the reaction. A similar solid medium was ineffective since *L. monocytogenes* did not grow on solid medium containing methylene blue. This finding has been reported by others. Dias and da Silva (50) made use of the fact that *L. monocytogenes* reduced 2,3,5-triphenyltetrazolium chloride to a bright-red formazan while *E. insidiosa* did not. Cells of *L. monocytogenes* in contact with the salt for 2 hr developed small red granules at the poles or center. Since, according to Gray et al. (83), some rough cultures of *L. monocytogenes* failed entirely to reduce the salt, or reduced it to only a very slight extent, the method would be effective only for smooth cultures. Gray et al. (83) made no mention of reddish granules in cultures grown in tetrazolium broth, or on agar containing tetrazolium. However, many cultures, when transferred to Tryptose Agar without the salt, still developed red colonies. Sometimes the color persisted for several transfers.

Splitting of Prontosil (4-sulfonamido-2′,4′-diaminobenzene) by *L. monocytogenes*, indicated by discoloration of a red medium, was employed by Kujumgiev (139). *E. insidiosa* had no action on this compound. The splitting was effective only in fluid medium. The saccharose indicator plate recommended by Kröger (138) as a rapid means for detecting colonies of *L. monocytogenes* was used by Ewald (89), since colonies of *E. insidiosa* remained colorless. Fraser (65A) reported that *L. monocytogenes* produced a diffusible substance which potentiated the lytic effect of *Staphylococcus* β toxin on sheep blood; *E. insidiosa* did not.

Biological Methods

Inoculation of mice. In an effort to overcome difficulties of isolating *L. monocytogenes* on artificial media, a number of investigators have suggested the so-called "biological method": inoculation of mice or other laboratory animals with suspected material. The method has not been accepted widely in the USA or Western Europe. However, it has been employed successfully for many years in the Soviet Union (93, 127, 132, 134, 153, 183, 184, 200, 249). By its use, the bacterium

has been recovered not only from human and animal sources but also from *Ixodes* sp. ticks (134, 237, 183), and stream water and crustaceans (184, 240). Sandvik et al. (227), in Norway, isolated the bacterium from sheep feces in a pen where lambs were affected with listeric septicemia. They inoculated fecal emulsion into white mice. In the United States, Gray (88) isolated *L. monocytogenes* from all of 35 mice inoculated intraperitoneally with distilled water extracts of silage thought to have incited an outbreak of listeric encephalitis in a flock of sheep. In this way, the bacterium's presence in the silage was established in three ways. In contrast, *L. monocytogenes* was also isolated on tryptose agar directly from the extracts, but only after they had been refrigerated for 10 days or more.

A number of valid objections to the use of mice must be admitted. There is a possibility that the animals might be carriers. *L. monocytogenes* has been isolated occasionally from various species of wild mice (11, 134, 183) but, as far as is known, never from laboratory mice. Olson et al. (180) found no particular advantage in the method. Rather, they found it less effective than conventional culture methods with several different media. It has been observed in a number of laboratories that mice inoculated subdurally with brain tissue from rabies suspects sometimes die from listeric infection (89). March (154) encountered this situation during an outbreak of rabies among cattle and suggested that subdural inoculation of mice might have considerable advantage over the usual culture methods for attempting to isolate *L. monocytogenes* from the bovine brain. However, his report revealed no convincing support for such a procedure. It is also known that mice inoculated with suspected material containing only a few organisms fail to develop clinical signs suggestive of active infection (63, 124, 146, 237). However, if the mice are sacrificed 5 to 15 days postexposure, *L. monocytogenes* can often be isolated from the liver or spleen, or both. Recently, Seeliger and Plab (237) showed that 4.6×10^6 organisms inoculated intraperitoneally were required to consistently kill Agnes Blum mice of 25 to 30 g. If the number dropped below this, chronic, inapparent infections were established, with the bacterium persisting in the liver for at least 3 months. Although mice may not necessarily be killed by inoculation of suspected material, they may, nevertheless, harbor the bacterium. Hence, they should be sacrificed and the liver and spleen cultured. Although mice appear to be the most susceptible of the easily obtained laboratory animals, guinea pigs could be used, but they are much less sensitive to exposure. Chinchillas are highly susceptible and, were it not for the expense, could serve the purpose. According to Dunaeva (55), the steppe lemming (*Lagurus lagurus, Pall.*) is the ideal animal for attempts to isolate *L. monocytogenes*, since it can be fatally infected with fewer than 10 organisms.

In spite of the disadvantages, there is justification for re-evaluation of the "biological" method, and its use should be encouraged until more reliable results can be obtained with nonliving media.

Inoculation of embryonating eggs. It has been known for many years that chicken embryos are highly susceptible to infection with *L. monocytogenes*. This fact has led several investigators to suggest using eggs in attempts to isolate the bacterium (45, 53, 58). Dontenwill and Knothe (53) claimed that the conspicuous necrotic foci that develop on the chorioallantoic membrane 48 hr after exposure of 10-day embryos are almost pathognomonic. The bacterium can be isolated easily from these eggs. As far as is known, the method has not been employed with material of human origin, but has been used successfully to isolate *L. monocytogenes* from spinal fluid of sheep with listeric encephalitis (58) and from a chicken brain (10). This facet, too, may be deserving of further investigation until methods of isolation are improved.

Characteristics Aiding Identification of Suspect Cultures

Several rather consistent and distinctive characteristics of *L. monocytogenes* are useful in identification of suspected cultures.

Motility. Most strains display rapid, characteristic tumbling motility when incubated at 18 to 20 C; this feature is usually absent at 37 C.

Monocyte production. The characteristic from which *L. monocytogenes* derives its species name is its ability to produce marked increase in numbers of circulating monocytes in most species of naturally and artificially infected monogastric animals. In rabbits inoculated intravenously, it is not uncommon to find monocytosis of 40% or more 3 to 5 days after exposure. Occasionally, a peak of 80% may be reached if slightly sublethal doses are given. If the dose is large, the animals often die before maximal monocytosis develops. The latter is most striking when the number of organisms produces severe illness, but not death. Maximal monocytosis can usually be accomplished by a 0.5-ml intravenous injection of distilled water suspension of the bacterium standardized to tube 1 of the McFarland nephelometer. Necropsy of the animals reveals that the liver,

and occasionally spleen or lungs or both, are completely studded with small, gray-white necrotic foci. The bacterium can be isolated from the viscera without difficulty. When evaluating large numbers of cultures for pathogenicity, mice or guinea pigs, but not rats or hamsters, may be used with satisfactory results.

Conjunctival instillation or Anton test. When cultures of *L. monocytogenes* are instilled into the conjunctival sac of rabbits, guinea pigs, or mice by means of a Pasteur pipette without scarifying the conjunctiva, a marked purulent conjunctivitis usually develops in 24 to 36 hr. This reaction was discovered independently and almost simultaneously by Anton (5) in Austria and Morris and Julianelle (161) in the United States. It is generally known as the "Anton reaction" even though Julianelle and Pons (118) were first to suggest this specific reaction as a rapid means of identifying suspect cultures. It is most striking and develops most consistently in the rabbit. The purulent reaction is usually at its peak about 3 days after exposure. After several days, the amount of exudate diminishes, revealing a markedly inflamed conjunctiva and opaque cornea. The inflammation and opacity persist for several days, and healing usually appears to be complete after 1 month. When the reaction is very severe, corneal opacity may persist for several weeks or even months (64). In nonpregnant animals, it appears to be a strictly localized reaction, and the animal shows no evidence of generalized infection. However, if the animals are treated with cortisone, death due to a listeric septicemia usually follows in 5 to 6 days (85, 89). If carried out on pregnant animals, abortion usually follows in 3 to 5 days (84, 159). Although the fetuses are fatally infected, the dam usually shows little evidence of generalized infection.

Although these tests may not be employed effectively with all recently isolated cultures, they are sufficiently dependable to be of value in confirming the identification of suspect cultures.

Detection by Fluorescent-Antibody (FA) Techniques

The difficulty and delay that may be encountered in attempting to isolate *L. monocytogenes* make it imperative that FA techniques be developed for quick detection of the bacterium, especially in meconium of newborns and spinal fluid of neonates where prompt treatment is essential if the patient is to survive. The technique could also be applied to cervical swabs from women with histories of habitual abortion. Smith et al. (245) have published FA studies with cultures of the bacterium. They encountered no particular difficulty in producing specific stains capable of distinguishing *L. monocytogenes* from other pathogens. This work was extended (247) to identifying the bacterium in experimentally infected animal tissues. Eveland (57) demonstrated *L. monocytogenes* in the spinal fluid of a patient by the FA technique. Of particular interest was the retrospective diagnosis of listeric infection in man by FA techniques (20, 166, 273) in cases in which specimens were not available for bacteriological diagnosis. Thin sections were cut from formaldehyde-fixed, paraffin-embedded tissues, deparaffinized and treated with serotype-specific FA. After treatment, fluorescing bacterial cells were observed in the tissues. Further research on the use of FA as an aid in the diagnosis of listeric infection is essential if diagnosis is to be made quickly and accurately in cases in which the bacterium will not grow initially in culture on nonliving media. The progress of this research has been reviewed recently by Cherry and Moody (36).

A scheme of the several methods for attempting to isolate *L. monocytogenes* is shown graphically in Fig. 1. Each has advantages and disadvantages and, until a simple but effective method is developed, the true incidence of *L. monocytogenes* infection will remain obscure.

PATHOGENICITY FOR LABORATORY ANIMALS

Male or Nonpregnant Animals

Some, in a moment of frustration, have lamented that Koch's postulates have done more to impede progress in clarifying the pathogenicity of bacterial infections than any other single thing. Those who have attempted to reproduce listeric encephalitis in large or small laboratory animals will eagerly join this lamentation. In fact, they would exclaim that for all his wisdom Solomon made a grievous omission when he confessed among his Proverbs: "There be three things which are too wonderful for me, yea, four which I know not: The way of an eagle in the air; the way of a serpent upon a rock; the way of a ship in the midst of the sea; and the way of a man with a maid." Had Solomon not been so preoccupied with his many wives, or had he been born a bit later, he certainly would have added the way of *Listeria monocytogenes* with a ruminant!

Attempts to produce encephalitis. Until a few years ago, it was virtually impossible to reproduce listeric encephalitis under laboratory conditions. Regardless of route of exposure, the animal, whether ruminant or monogastric, usually died from septicemia with or without associated meningitis, but not from localized encephalitis as

seen in most field cases. Inconsistently, some investigators succeeded, but then could not repeat their findings. Best results seemed to follow intranasal exposure or injection into the carotid artery. Occasionally, rabbits, mice, pigs, and goats exposed by conjunctival instillation developed encephalitis (7, 75, 78, 85), but at best it was a chance occurrence.

Gray et al. (81), in a study devoted to the effect of chlortetracycline on laboratory-produced listeric infections, stumbled on a trail that unexpectedly led to serendip. When rabbits inoculated intracerebrally were treated intravenously with chlortetracycline (2.5 mg/lb of body weight) an equilibrium was established which permitted multiplication of the bacterium in the brain but not in blood or viscera. Four to six days after exposure, these animals developed signs, and sometimes lesions, simulating those seen in ruminants. It may be questioned what this contributed to a better understanding of the pathogenesis of the disease, since obviously ruminants are not exposed naturally by large numbers of bacteria introduced intracerebrally followed by mysterious intravenous injection of chlortetracycline. But it was a useful tool at the time. It might even be tried in studies on other infections of the central nervous system, if such unrealistic methods are necessary to fulfill Koch's postulates. But this could lead to a long digression on some of the nonsense methods used to produce so-called "infections" in laboratory animals. Before more wrath is meted out by a long-suffering editor, let it be quickly added that it does not seem like much of an achievement in pathogenesis or anything else—an exception that validates this is virulence titration of a culture—to pump an animal full of bacteria in an utterly unnatural way and then boast that the disease has been artificially reproduced or that Koch's postulates have been fulfilled.

Olson et al. (182) found a more realistic approach in producing encephalitis in ruminants. From the blood of sheep with high temperatures among flocks actively infected with *L. monocytogenes* and from lymph nodes and blood from cattle with bovine mucosal disease, they isolated a nonbacterial listeriosis-enhancing agent (LEA). Intranasal exposure of sheep with a combination of LEA and *L. monocytogenes* resulted in listeric encephalitis in more than 80% of exposed animals. Results were less spectacular in calves. The mode of action remains unexplained, and its role in field outbreaks is well concealed within the still tangled snarl of information relating to pathogenesis of listeric infection. But this discussion of laboratory-induced infections is rapidly becoming contaminated with pathogenesis. Perhaps it should be streaked out on a new paragraph lest contamination becomes too great.

General routes of exposure. Although Koch's postulates cannot be fulfilled with listeric encephalitis of ruminants, they can be fulfilled rather easily for listeric septicemia of monogastric animals. Intravenous inoculation of almost any monogastric laboratory animal leads to rapidly fatal septicemia with or without associated meningitis. There is considerable disagreement as to whether animals exposed by this route develop meningitis. The result is undoubtedly a function of the interplay of several factors, such as size of inoculum, virulence of culture, and condition of the test animal. Pathogenicity studies with *L. monocytogenes*, as with almost all other bacteria, are confounded by a deplorable assemblage of meaningless jargon. One is constantly plagued by such exacting gems as "0.5 ml of a 24-hr broth culture," "0.2 ml of a dense broth culture," or "the growth of a 24-hr agar slant was washed off with saline and 0.2 ml injected intraperitoneally." As long as microbiolo-

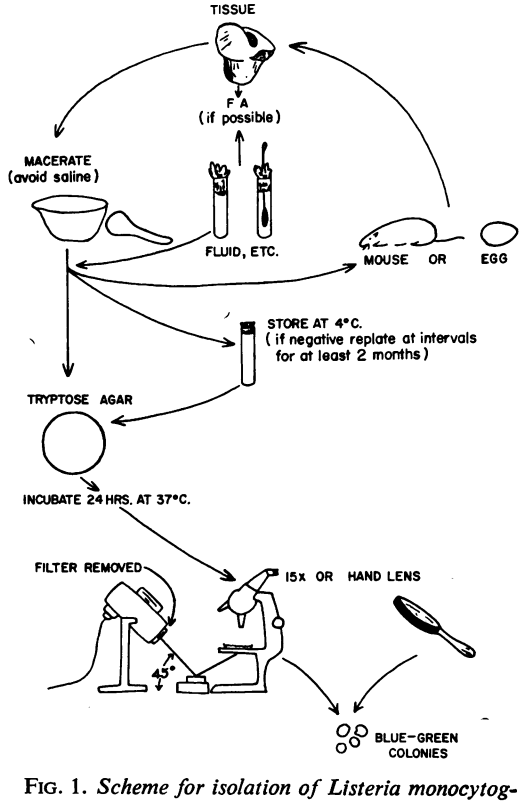

FIG. 1. *Scheme for isolation of Listeria monocytogenes.*

gists persist in this peculiar practice of expressing dosage in obscurities rather than definitely titrated amounts, results obtained in different laboratories can never be compared. In our laboratory, meningitis has been a rare finding in intravenously exposed rabbits (0.7-ml distilled water suspension standardized to McFarland nephelometer tube 1) and intraperitoneally exposed mice (indefinite number of bacteria in silage extracts; 88). Death usually results from septicemia. This termination does not necessarily contradict those which report meningitis as a consistent finding. The dose might be quite different from ours.

Unless given an overwhelming number of organisms, most intravenously exposed monogastric animals develop marked monocytosis.

Intraperitoneal inoculation readily results in fatal septicemia in rabbits and mice. No definite information is available on number of cells required to infect rabbits, but the LD_{50} for mice has been determined by a number of investigators. It ranges from 10^3 (124) to 4.6×10^6 (237) cells, depending on strain of mice employed and virulence of culture. It is difficult, and some claim impossible, to infect guinea pigs by the intraperitoneal route. Kautter et al. (124) gave the LD_{50} as greater than 10^7 cells.

At necropsy, listeric septicemia is characterized by accompanying focal necrosis. Usually most conspicuous are the foci of the liver, which may also be numerous in the spleen, lungs, adrenal glands, tonsils, and sometimes the intestinal tract. In guinea pigs, the foci are often seen only on the myocardium, a rare finding in other mammals but common in birds. Often the peritoneal cavity contains large amounts of clear or slightly turbid brownish-yellow fluid which, according to Murray et al. (164), contains large numbers of triple-phosphate crystals. If the animal develops meningitis, the meninges may be studded with similar foci. Histologically, the lesions in all organs consist of more or less well-defined areas of focal necrosis characterized by infiltration of mononuclear cells. At times, there is marked infiltration with polymorphonuclear leukocytes giving the appearance of a purulent reaction.

Usually little difficulty is encountered in attempting to isolate *L. monocytogenes* from the liver or spleen of animals that die with septicemia. It may be necessary only occasionally to resort to maceration and refrigeration of material. In contrast, it is rather difficult to isolate the organism from blood of these animals either ante- or postmortem. This observation has been recorded repeatedly since it was first emphasized in the original report of Murray et al. (164), and remains unexplained today.

With the exception of white mice (45, 118, 146, 207) and chinchillas (85, 235), oral exposure, even of massive numbers of organisms, has little effect on most nonpregnant adult animals, but very young animals may develop fatal septicemia (84, 95, 164). Even though most orally exposed animals fail to develop clinical signs suggestive of infection, they may harbor the bacterium for relatively long periods. Osebold and Inouye (186) found and Gray et al. (84) confirmed that, although these animals have no lesions when sacrificed and initial cultures from the viscera fail to reveal the bacterium, it can be isolated quite consistently if tissue suspensions are refrigerated for several weeks, this finding indicating that the animals may be healthy carriers.

Both Laymann (146) and Popov (207) mentioned that orally exposed mice developed nervous signs more often than mice exposed by other routes. If this is actually true, it suggests that mice may be somewhat distinctive in several respects, since the finding is a rare one among other laboratory animals.

Since *L. monocytogenes* is often associated with infection of the central nervous system, numerous investigators have employed intracerebral inoculation of a variety of laboratory animals ranging from mice (124) to cows (75). Although an acute fatal meningitis or encephalitis, or both, with or without associated septicemia, are produced, the infection does not simulate that seen in either listeric encephalitis of ruminants or meningoencephalitis of monogastric animals and man. Kautter et al. (124) found that mice could be infected by as few as five organisms by the intracerebral route, whereas 10^3 to 10^5 cells were required for infection via the respiratory tract and 10^3 to 3×10^4 cells by the intraperitoneal or intravenous route.

The respiratory route is often mentioned as a possible means of exposure. Aerosols were used to expose mice, guinea pigs, and rabbits by Urbach and Schabinski (271), mice by Dedié (45), mice, guinea pigs, and young swine by Popov (207), and mice, hamsters, guinea pigs, rabbits, and monkeys by Kautter et al. (124, 124*a*) and Silverman et al. (241*a*). Although each used different methods for producing aerosols, different methods of expressing dosage, and different lengths of exposure, all agreed that aerosol exposure produced more lethal effects than other routes of exposure. Urbach and Schabinski (271) used Shope's method for exposure. Their animals died in 3 to 6 days from bronchopneumonia without developing lesions of the liver or spleen. The other authors all reported deaths due to typical listeric septicemia. No mention was made of

nervous signs or alterations in the central nervous system.

Popov (207) found little difference in susceptibility between mice and guinea pigs but found young swine quite resistant to aerosol exposure. Only when 30 ml of suspension containing 20 billion cells per milliliter was diffused over a 5-hr period of two successive days could swine be infected. In contrast, 70% of exposed mice were fatally infected by 2-min inhalation of aerosol containing somewhat less than 250 million cells. A 30-sec exposure killed 60% of the mice while 15-sec exposure was without apparent effect. Kautter et al. (124) found mice to be considerably more susceptible than guinea pigs. Although monkeys could not be killed by this procedure, they developed transitory febrile reaction with increase in antibody titer. They also found aerosol exposure to be more consistently reproducible than either intravenous, intraperitoneal, or intracranial inoculation. These studies indicate that the aerogenous route may play an important role in natural infections in both animals and man.

As mentioned under *Characteristics Aiding Identification of Suspect Cultures*, conjunctivitis can be produced in several laboratory animals by instilling culture in the conjunctival sac. This phenomenon has been studied most extensively by Anton (5), Morris and Julianelle (161), Julianelle and Moore, and Flamm and Zehetbauer (64). In addition to the gross lesions previously described, the conjunctiva and cornea are infiltrated with monocytes. After the fifth postexposure day, the proportion of polymorphonuclear leukocytes increased until, by the tenth day, these cells predominated, giving the appearance of a purulent reaction. Congested capillaries, some of which showed proliferation of the endothelial lining, gave the appearance of pannus of the cornea (161). Occasionally there was hyperemia of the iris. At the height of infection, there was marked edema of the involved area. Usually the deeper structures did not become involved and the lesion was confined to the eye. Some animals showed mild monocytosis. The exposed eye developed relatively long-lasting local immunity without development of general immunity. However, immunity resulting from other routes of exposure does not protect the eye. Some animals may relapse after either spontaneous recovery or topical treatment with antibiotics. Infrequently animals exposed in this manner developed septicemia or in a few instances meningitis or meningoencephalitis (7, 75, 78, 85).

The early observation of Bloom (26) that subcutaneous inoculation of *L. monocytogenes* produced somewhat distinctive abscessation lies almost completely neglected beneath gathering dust of lonely library stacks entrusted with the wearisome obligation of supporting 1928 volumes. Gray et al. (83) used the method described on the yellowing pages in studies on comparative virulence of smooth and rough cultures of *L. monocytogenes*. In view of the well-established fact that many human patients, particularly newborn ones, develop listeric skin lesions (89, 136, 235), plus the recent reports of skin lesions developing on the hands and arms of veterinarians after handling infected aborted bovine fetuses (51, 120, 189), and skin lesions due to *L. monocytogenes* of more obscure origin (157, 173), perhaps such lesions should be investigated further.

Subcutaneous inoculation of rabbits with *L. monocytogenes* produced a rather ordinary inflammatory reaction followed by formation of definite abscesses. The latter persist for several days; the center becomes necrotic, and in most instances they eventually slough away with or without formation of scars. It appears to be local reaction with no indication of generalized infection. However, there is marked increase in circulating monocytes, and Bloom (26) used this reaction to study the origin of monocytes. Gray et al. (83) found that, when 15 TR units of hyaluronidase per ml of culture was added to the bacterial suspension, the local lesion was less marked. However, when the animals were sacrificed, small foci of necrosis were observed in a wide area (up to 10 cm) surrounding the inoculation site, both in the skin and adjacent muscles. They suggested that this lesion might have interesting possibilities for studies on S to R or R to S dissociation.

Laymann (146) reported development of septicemia in nonpregnant mice inoculated subcutaneously, and Potel (84) produced abortion in pregnant mice by this route. However, no mention was made of necrotic lesions at the inoculation site. Perhaps it reflects another distinctive characteristic of the mouse.

Occasionally, it has been observed in this laboratory that, when intravenous inoculations inadvertently find their way into the adjacent tissue, small abscesses are formed which may persist and contain viable bacteria for periods of several months. Perhaps it has been observed also in other laboratories. If so, they apparently have been unwilling to admit in print that their technicians make mistakes.

From all the travail and pains of labor expended in exposing animals to artificially induced listeric infections, comparatively little has been brought forth, except perhaps that the only form of naturally occurring listeric infection which was consistently reproducible in nonpregnant laboratory animals is septicemia. This has done

much to impede progress toward clarifying the pathogenesis of other forms of listeric infection, particularly the important meningitic and encephalitic forms.

Choice of laboratory animal. Although a number of different animals may be used to produce artificially induced listeric infections, the rabbit is perhaps ideal from the standpoint of both susceptibility and size. The mouse may be equally susceptible, but has certain disadvantages in size and considerable variation in susceptibility among the various genetic strains. Guinea pigs have been used with some success, but are considerably less susceptible than rabbits or mice. Rats have not been used widely, and the few investigators who worked with them found them to be quite resistant. However, Gray (85) rather consistenty produced fatal septicemia in rats exposed by conjunctival instillation accompanied by injection of 3 mg of prednisone per 100 g of body weight for 4 days. The few investigators who employed hamsters found them to be somewhat resistant. Nordland (170) induced infection when exposure was accompanied by administration of 5 mg of cortisone per day. Cats and dogs are extremely resistant. As mentioned under *Biological Methods for Isolation*, chinchillas are highly susceptible but their use is limited by their expense and, judging from personal experience, their unfriendly attitude toward laboratory personnel. The steppe lemming (*Lagurus lagurus*) which Dunaeva (55) claimed can be infected fatally by one bacterial cell is not available in the USA. Recently, Welshimer and Winglewish (281*b*) found that the American vole (*Lagurus curtatus*) could be killed in 120 hr by intraperitoneal injection of 23 to 25 cells of a culture of *L. monocytogenes* of which an injection of 10^5 cells was required to kill 16- to 20-g Swiss Webster Rockland Farm mice in 5 to 7 days. If the vole can be reared successfully in captivity, it may prove highly useful for studies on artificially induced listeric infection.

Use of birds. Several species of birds have been used as laboratory animals for studies with *L. monocytogenes*. These include the chicken, turkey, pigeon, wood grouse, sparrow, finch, and canary. The latter two appear to be especially susceptible. In general, most birds are difficult to infect, and large numbers of organisms are required to produce death. Segre et al. (86) even attempted to alter the tricarboxylic acid cycle by means of antimetabolites, but still failed to incite infection in 1- to 2-week-old chicks. When infection did succeed, death was due to septicemia characterized by myocardial degeneration with focal or diffuse necrosis, providing the birds lived for 5 to 7 days. Necrotic foci similar to those seen in monogastric animals may be seen at times on the liver or spleen, or both, or other viscera. The lesions are similar, if not identical, to those seen in uncomplicated naturally induced infections in birds. The lesions are described under LISTERIC INFECTION OF FOWL.

If embryonating chicken eggs may be considered to be laboratory animals, their susceptibility is in inverse proportion to their small size, small expense, and small maintenance requirements, as already hinted under *Biological Methods for Isolation* (45, 53, 195). Their use could save considerable money on purchases of expensive media, since Segre et al. (86) found that 1.8 cells constituted an LD_{50} in 7-day embryos, 11 cells for 14-day-old embryos. Numerous discrete white foci on the chorioallantoic membrane represented the most consistent and distinctive lesion. There may also be thickening of the ectoderm in addition to lesions of the endoderm and mesoderm. In embryos older than 10 days, there may be focal hepatic necrosis. Lesions in the brain have not been reported. However, Mencikova (157) found that 18-day-old embryonating chicken eggs inoculated lightly on the chorioallantoic membrane hatched and developed normally, although *L. monocytogenes* could be isolated from tissues and feathers of the chicks. However, when large inocula were employed, the chicks hatched normally but developed encephalitis and died within 5 days after hatching. It is not unlike what may be true in some prenatally infected infants as will be suggested a few sections later, or what has been observed in prenatally infected rabbits as will be revealed in the next section.

Pregnant Animals

Although Burn (32) isolated *L. monocytogenes* from a human infant as early as 1933 and Graham et al. (75) in 1939 recorded listeric abortion in cows followed by Paterson's (194) report of a similar condition in sheep 1 year later, scant attention was given to perinatal listeric infection prior to 1950, and even less attention was accorded laboratory reproduction of this condition.

Perhaps the first deliberate studies on artificial infection of pregnant animals with *L. monocytogenes* were undertaken by Hindmarsh and Blumer in 1932 (103). They isolated a gram-positive rod from a ewe and premature lamb during an outbreak of abortion among sheep. When the organism was inoculated intravenously, intraperitoneally, or swabbed into the vagina of pregnant guinea pigs, abortion followed, and the bacterium was recovered from the fetuses and vaginal discharge. A pregnant ewe inoculated

intravenously also aborted, and the bacterium was isolated from the placenta and uterine wall. The authors called this bacterium a "diphtheroid bacillus." Unfortunately, it was never further identified, but almost certainly was *L. monocytogenes*.

Previously, Anton (5) unintentionally produced death in a rabbit pregnant 1 week and abortion in another late in gestation, following conjunctival instillation of *L. monocytogenes*. Belin (16) instilled *L. monocytogenes* into the conjunctival sac of a pregnant guinea pig and it aborted several days later. However, these authors were interested primarily in the conjunctivitis, and no particular significance was attached to the abortion. In view of the events of the past 10 years which demonstrated emphatically the important role of *L. monocytogenes* in perinatal infections, plus the fact that some rabbits from which Murray et al. (164) isolated the bacterium were pregnant, it is somewhat of a conundrum that interest in artificially induced perinatal infections came in a tardy manner.

During the late 1930's, Graham et al. (75) produced abortion in a 5-month-pregnant heifer exposed intravenously to *L. monocytogenes*, while Paterson (194) reported no reaction in pregnant sheep dosed orally with serum broth cultures of the bacterium. There is no record of further attempts to produce listeric abortion under laboratory conditions until the almost simultaneous but independent studies of Potel (84) on guinea pigs and mice, of Hahnefeld and Hahnefeld (95) on rabbits and goats, of Osebold and Inouye (186) on rabbits, and of Gray et al. (84) on rabbits, goats, sheep, and a cow. Subsequent studies on pregnant rats were reported by Payne (198) and by Schultz (234), on rabbits by Suchanova et al. (259), on cows by Osebold et al. (188), on sheep by Mollelo and Jensen (159*a*), and on rabbits by Miller and Muraschi (159). Exposure included such realistic routes as oral (84, 95), conjunctival (84, 159), and intravaginal (84, 186), and such unrealistic ones as intravenous (188, 159*a*), intraperitoneal (198), via the vena cava or aorta (234), and intraperitoneal implantation of collodion sacs containing the bacterium (259). It must be admitted that some investigators who used unrealistic methods were not concerned primarily with mere production of abortion, but rather in the general mechanism of abortion, and they presented well-defined reasons for employing the methods chosen (198, 234, 259).

With sufficient exceptions to make a valid rule (84, 198) regardless of animal species, stage of gestation, type of placenta, or route of exposure, the uterine contents quickly became infected. If exposure were early in gestation, the conceptus was aborted; if near term, the fetus was either stillborn or the young survived only a few days. If the uterine contents were expelled quickly and completely, the dam usually displayed few signs of generalized infection. However, when fragments of the infected conceptus were retained, the dam often developed a severe necrotic metritis which usually terminated in fatal septicemia. Although most dams developed only relatively mild indications of infection, the young were invariably fatally infected. Of more than 150 full-term young rabbits born during the studies of Gray et al. (84), only one survived more than 8 days.

Monogastric animals. Gray et al. (84) and Hahnefeld and Hahnefeld (95) reported almost identical results with orally exposed pregnant rabbits and goats. The same was true for conjunctival exposure of pregnant rabbits reported by Gray et al. (84) and Miller and Muraschi (159), indicating that the results of exposure are highly reproducible. It also indicated that the gravid uterus is highly vulnerable to insult by either of the channels of infection, that the gravid uterus is highly susceptible to infection with *L. monocytogenes*, that the uterine contents are primary foci of infection, that only small numbers of organisms are required to initiate infection of the pregnant uterus, and that conjunctivitis which develops from conjunctival instillation of *L. monocytogenes* is not a local reaction as often supposed.

A rough sketch of a typical reaction would show a slightly depressed doe with thick, blood-tinged, mucuslike vaginal discharge containing large numbers of the bacterium at 2 to 4 days after exposure. Abortion or birth of infected young, depending on stage of gestation, follows shortly. Aborted fetuses, even litter mates, may be well-developed and intact or badly macerated masses. Lesions usually are confined to the liver, which is mottled and pale yellow, but focal necrosis is rare. Young born at term are normal in size and development. If born alive, they appear acutely ill and whine as if in pain. In spite of these signs, most nurse until death. At necropsy, the stomach is found to be filled with milk and the liver completely studded with necrotic foci. Sometimes, there are foci in other viscera. Young which survive more than 5 days may develop signs suggestive of meningitis or meningoencephalitis. Amniotic fluid is blood-tinged or contains large flecks of yellowish puslike material, or both. Cotyledons are hemorrhagic or necrotic and may show small areas of necrosis. The doe appears depressed for a short period after parturition but is seldom acutely ill. Occasionally, preg-

nancy may be interrupted as early as 24 hr after exposure, suggesting that the bacterium very quickly crosses the placental barrier. The picture simulates in almost every detail listeric infection in human neonates and helps to re-establish one's faith in Koch's postulates.

One of the most significant contributions to emerge from these studies was the observation by Gray et al. (84) and Miller and Muraschi (159) that some apparently healthy kindlings, born to infected does, developed meningitis during the neonatal period. Sometimes these signs did not appear until 3 weeks after birth to does exposed by conjunctival instillation (159). This response strongly supports the contention that low-grade perinatal infections may pass undetected, and that infants which develop listeric meningitis early in life are actually exposed *in utero* or during birth rather than by environmental contact during the neonatal period.

Rebreeding and extra-uterine infection. Gray et al. (84) were not content merely with a facsimile of prenatal infection but extended their studies into its effect on subsequent reproduction capability of the doe and also to the possibility of extra-uterine perinatal infection. They encountered not only simulated, naturally induced perinatal infection but anticipated some things that had yet to be observed in natural infections, i.e., habitual abortion and the possibility of mental retardation in later life resulting from inapparent infection at birth.

When does which had given birth to infected litters were rebred as early as 9 days postpartum, most produced healthy full-term litters, indicating normal reproductive function. Although a few litters were stillborn or abandoned, there was no evidence to incriminate *L. monocytogenes* in these disorders. It was not determined whether some does which appeared to be pregnant but failed to produce litters were merely pseudopregnant, which is known to occur in rabbits, or whether there occurred implantation with early intra-uterine death and complete resorption of the conceptus as observed by Osebold and Inouye (186) following intravaginal instillation early in gestation. Nevertheless, some does aborted spontaneously during the third week of gestation and *L. monocytogenes* was isolated from the aborted conceptus. A doe aborted during three consecutive gestations and the bacterium was isolated from the fetuses and vagina of the doe after each abortion. Further attempts to rebreed this individual over a 9-month period failed. When sacrificed, necropsy revealed numerous adhesions surrounding the reproductive system and marked fibrosis of the uterus. This observation established not only the possibility that *L. monocytogenes* might persist in the female genital tract for relatively long periods with no indication of illness, and initiate infection during several succeeding gestations, but also that such a series of abortions may lead to sterility. This possibility was further supported by findings at necropsy of apparently healthy does sacrificed at varying periods postpartum. Lesions were confined generally to the reproductive system; in most, there was normal involution with only mild necrotic endometritis. However, in others the uterus appeared normal except for abscesses which ranged from 2 to 22 mm in diameter; the bacterium could be isolated only from these abscesses. It is conceivable that similar persistent abscesses constituted the source of infection for the next gestation. Although unconfirmed, it is possible that a similar condition may exist in women, and this finding has given strong support to those who hold that *L. monocytogenes* may be responsible for some cases of so-called habitual abortion, as discussed under that section.

When does which had given birth to infected litters were rebred and re-exposed, they again aborted or delivered infected young as they did during initial exposure. Some aborted in spite of agglutinating antibody titers of 1:1,280, indicating that intrauterine listeric infection gave little or no immunity against similar subsequent infection (84). In contradiction, Schultz (234) observed apparent immunity to reinfection by rats which had given birth to infected litters. It is also possible that this refractoriness is merely further reflection of the rat's inherent resistance to listeric infection.

Gray et al. (84) also succeeded in producing extrauterine neonatal infections either during birth or by way of the does' milk. When *L. monocytogenes* was instilled into the vagina of pregnant rabbits several hours before term, the newborn kindlings' skin was often highly contaminated with the bacterium. Sometimes, it was also isolated from mouth swab cultures. Although relatively few kindlings were actually infected by this method, some died from listeric septicemia during the first 3 to 8 days of life. Others which survived for longer periods often showed signs suggestive of central nervous system disorder and some died from listeric meningoencephalitis.

If kindlings born to noninfected does were suckled by infected does or if the bacterium was placed in the does' drinking water a few hours post partum, some kindlings developed either septicemia or meningoencephalitis. With the known difficulty of transmitting listeric infection by contact alone, these young appeared to be in-

fected through the does' milk. Although *L. monocytogenes* was isolated from the milk of some sacrificed does, from mouth swabs of kindlings immediately after suckling, and from stomach contents of sacrificed kindlings, it was never directly isolated from the milk of living does. However, *L. monocytogenes* has been isolated directly from milk of other animals which present fewer milking difficulties than do rabbits. Other animals included orally exposed goats (84, 95) and sheep (84, 194), and intravenously exposed cows (188) and a sheep (45). The bacterium has also been isolated after death from the milk of artificially infected guinea pigs by Potel (84) and from rabbits (95, 159). Also, *L. monocytogenes* has been isolated a number of times from milk of naturally infected cows (85, 89, 188, 223, 275, 285). All of the facts make it appear quite probable that the bacterium can be transmitted to a suckling from an infected mother. Hahnefeld and Hahnefeld (95) reported no apparent effect on either kindlings or doe when the doe was exposed orally immediately postpartum.

This series of studies not only demonstrated that the uterine contents of pregnant rabbits are highly susceptible to listeric infection, but also that such infections may persist and serve as a source of infection for the next pregnancy. It also demonstrated that some young animals may experience inapparent infections which could be detected only by isolation of *L. monocytogenes* from the urine or the liver, spleen, and brain of kindlings sacrificed during the first 2 weeks of life. In some, the liver or spleen showed infarcts of varying size, and the bacterium was isolated only from the infarcts, indicating that lightly exposed newborn rabbits may experience low-grade infection and survive. These findings support the suggestion of Lang (144) that some children with mental deficiencies of unknown etiology may have experienced inapparent or undetected listeric infection during the perinatal period. Some young rabbits observed by Gray et al. (84) had mild signs of central nervous system disturbance for several weeks, with apparent recovery. Physically, these animals developed normally but, in the absence of suitable tests, there was no measure of mental development. It is conceivable that this may also occur in human infants, and that retarded mental development may be a sequel. Retarded mental development has been reported as not uncommon after confirmed listeric meningitis in infants (89, 136, 235).

Limited studies showed oxytetracycline treatment of pregnant rabbits exposed late in gestation to be of some benefit in protecting unborn young (84). Also, some young born to does exposed a few days prepartum survived after oxytetracycline treatment, whereas untreated litter mates always died. Penicillin treatment of does which developed septicemia after delivery of infected uterine contents also gave some protection (159), even though penicillin is generally contraindicated in listeric infection (235). Studies should be expanded before conclusions may be drawn. Nevertheless, prompt and adequate treatment, preferably with tetracycline antibiotics, of either the mother or infected newborn may result in successful infant salvage when listeric infection is likely. The value of the regimen was subsequently confirmed by Hood (104).

Although pertinent studies have established that *L. monocytogenes* can produce abortion during the latter half of pregnancy, there was controversy until recently as to whether it could also attack embryonic tissue early in gestation. Gray et al. (84) found no detrimental effect after conjunctival, oral, or intravaginal exposure during the first week of gestation, or from intravaginal instillation of culture immediately before copulation, even though the bacterium could be isolated from the vagina for as long as 9 days. Also, rabbits which aborted spontaneously always did so only during the third gestation week (84). In contrast, Osebold and Inouye (149) incited infection during the second week of gestation by intravaginal instillation; Hahnefeld and Hahnefeld (95) reported similar results from oral exposure at this time of gestation. Recently, Gray (89) received for serological typing a culture isolated from a 2-month human embryo. It may be inferred that, although pregnancy interruptions due to *L. monocytogenes* are most common during the last trimester, they can occur at any time during pregnancy.

Ruminants. Oral exposure of pregnant sheep (84) and goats (84, 95) produced essentially the same results as in rabbits. The only record of oral exposure of a pregnant cow (84) resulted in birth of an apparently normal full-term calf. As would be expected, intravenous inoculation of pregnant cows readily produced abortion (75, 188).

In contrast to guinea pigs and rabbits, conjunctival exposure of pregnant goats produced only mild conjunctivitis of short duration followed by birth of apparently normal full-term kids (84). However, one goat developed fatal listeric encephalitis at parturition, and some of the others which appeared normal when sacrificed had histological alterations of the medulla oblongata. The lesions were characteristic of listeric encephalitis of ruminants. This observation strongly supports the views of those who hold that the bacterium may pass along branches of

the trigeminal nerve of naturally induced cases of listeric encephalitis (7, 29, 85, 182). The support is immediately shaken by the results of Osebold et al. (188), whose findings were essentially identical to those in an intravenously exposed cow sacrificed 19 days after abortion.

Failure to produce intrauterine infection of pregnant ruminants by conjunctival exposure may reflect basic differences between ruminant and monogastric animals. The fact that ruminants developed encephalitis by the conjunctival route of exposure without infection of the uterine contents may offer possible explanation for the oft expressed observation that listeric encephalitis in sheep is most prevalent at the time of the year when ewes are pregnant; yet listeric abortion in sheep is relatively rare. It strengthens the position of those who hold that the two manifestations of infection have a distinct pathogenesis.

Pathological changes of the reproductive tract. Another contribution made by these studies is the insight into what may take place in the uterus during natural infection, and its possible consequences. Since the mother is seldom acutely ill and death is rare in natural perinatal infection, investigators are completely dependent on laboratory findings for pertinent information. Perinatal infection is complicated somewhat by differences in placental structure of the various animal species. Thus, the projection of laboratory findings into natural infections has shortcomings.

When Payne (198) inoculated pregnant rats intraperitoneally and Schultz (234) inoculated them by way of the aorta or vena cava, or Suchanova et al. (259) implanted collodion sacs containing *L. monocytogenes* in the peritoneal cavity of pregnant rabbits, their concern was not so much with production of abortion as with the mechanism by which *L. monocytogenes* produced abortion. Payne (198) found that gross lesions were confined almost entirely to the placenta which contained numerous white foci or streaks of necrosis. When the placenta was severely affected, the fetuses were either moribund or dead and in process of resorption. The uterine wall was free from inflammatory change, but large numbers of the bacterium accumulated in the walls of the maternal artery which supplies the placenta. The predominant lesions occurred within the tissues of the junctional zone of the placental disc, causing large areas to become necrotic. Sometimes thrombi formed, resulting in infarction of the entire placenta. The infection frequently extended into the metrial gland and the labyrinth. The advancing edge was characterized by a band of polymorphs, tissue debris, and bacteria. Bacteria were also found in the trophoblsta cells and within the maternal blood spaces. Large numbers of monocytes and polymorphs were seen in the maternal sinuses. Degenerated fetal tissue left a mass of eosinophilic debris surrounded by polymorphs and bacteria. This type of infection was referred to as "descending infection" and, although it led to widespread destruction of placental tissue, fetal morbidity and mortalite were not necessarily associated with it. The fetuses were affected only slightly even by severe changes in the placenta.

Only when placental destruction was greater than that required for survival of the fetuses did their death appear to occur. There was no histological evidence of resistance to bacterial invasion, and the polymorphs appeared to be almost entirely of maternal origin.

Schultz (234) did not go into great detail on the actual alterations observed, but found that repeated or permanent flow of infected blood from the descending aorta brought many bacteria into the placenta. If the flow of maternal blood in the placenta was reduced or arrested, there was rapid accumulation of bacteria which greatly enhanced the hazard to the fetus. In his opinion, the maternal capillary network appeared to be the most effective barrier against the bacterium, since after inoculation into the vena cava, arterial blood was sterile when it reached the placenta. He claimed that rat blood contained an effective bactericide against *L. monocytogenes*. It may help to explain the rat's high resistance to listeric infection reported by those who have attempted to infect them artificially. Without citing specific comparisons, Schultz (234) felt that their studies helped to explain why diaplacental infection is relatively rare. He concluded that capillary filters and bactericidal substances of the mother are more protective than the placenta.

Suchanova et al. (259) isolated *L. monocytogenes* from some fetuses, even though the uterus or placenta showed no pathological alterations, suggesting that *L. monocytogenes* has an extraordinary affinity for placental tissue and that it can cross the intact placental barrier. It may result from anemic infarcts which appear during the last half of gestation. If so, it offers a possible explanation why listeric abortion is most common during this period of pregnancy. These authors also contend that there is a filterable form of the bacterium capable of crossing the intact placenta. The gross and microscopic lesions appeared to be essentially identical to those described for rabbits exposed orally or by conjunctival or intravaginal instillation (84, 95, 159, 186). Unfortunately, the histological findings of both Gray et al. (84) and Miller and Muraschi (159) remain unpublished.

However, they are recorded in a 1956 Michigan State University doctoral dissertation by C. Singh entitled, "Pathology and Bacteriology of Abortion and Perinatal Death of Young in Rabbits, Sheep and Goats Induced by *Listeria Monocytogenes*." The results appear to be identical to those of Hahnefeld and Hahnefeld (95), who gave a very good description in German. Although pregnant rabbits which died of listeric septicemia showed various degrees of metritis, ranging from marked congestion to extensive necrosis with partial resorption or mummification of the fetuses and containing large amounts of pus or caseous puslike material adhering to the endometrium, these alterations cannot be considered typical of natural infection. The information must come from apparently normal sacrificed animals.

The uteri of sacrificed rabbits showed marked congestion, caseous necrotic material on the endometrial surface with little to marked purulent exudate, or dry necrotic masses in the uterine cavity. The inflammatory process was centered at the site of placental implant, and the endometrium reddened and corrugated. Cotyledons were usually necrotic. Histologically, there was acute necrotic metritis; the endometrium was caseously necrotic, involving a limited area of the epithelial lining and lamina propria of the endometrium, and distention of the uterine glands with purulent exudate and bacteria. Gram-positive bacteria were seen on both maternal and fetal sides of the placenta. The adjacent fetal membranes contained purulent exudate. Unfortunately, there was no complete histological study to trace the entire course from delivery of infected young to complete recovery of the reproductive tract. However, 1 to 2 months postpartum, the uterus was still enlarged, the walls were thickened, and there was chronic endometritis with hyalinization and lymphocytic infiltration of fragments of placental tissue.

Limited information gathered from studies on ruminants gave essentially the same findings (84, 95, 188, 194), indicating that placental differences played no significant part in determining the course of infection.

If the above laboratory findings can be projected to natural infections, it gives some insight into what takes place during natural perinatal listeric infection. Regardless of animal species, there was general agreement that the placenta offered little or no resistance to invasion by the bacterium and that this was the primary site of active infection. The uterus was extensively involved only when dead fetal material was retained, but only slightly involved when the dead conceptus was aborted quickly or the early conceptus resorbed over a long time. Primary damage to the fetus appeared to result from placental destruction and resulting dysfunction, with actual infection of the fetus a secondary consequence. With few exceptions, there was no evidence that single listeric metritis had any long-lasting effect on the uterus or reproductive function, although it might shed or contain the bacterium for 1 month or more. This is supported by the observation that most animals which give birth to naturally infected young come into estrus normally, and subsequent pregnancy is without complication. The same is true for women who give birth to infected infants. It suggests that what was observed in the laboratory quite accurately simulates what must take place in natural infections. Barrow and Pugh had opportunity to confirm the finding when they delivered an infected infant by Caesarian section. Unfortunately, if they did notice changes, they failed to record them in their publication (J. Pathol. Bacteriol. **75**:9–16, 1958).

LISTERIC INFECTION IN MAN

Retrospect

L. monocytogenes continues its inconsistencies when attacking man. In contrast to animals, where it usually boldly attacks the finest and the strongest in the herd or flock, in man it usually attacks the very young, sometimes even before they are born, or the very old, or those already weakened by other disorders.

Seeliger (235) cited some 18 of the various forms of listeric infection. Their descriptions by different names are found in the medical literature previous to 1929 when Nyfeldt (174) recorded the first isolation of *L. monocytogenes* from a human subject. These isolations and those he made in the next few years are of interest not only historically, but also for their almost cruel irony. It was as if the bacterium sought revenge against him.

Nyfeldt (174) made his isolations from the blood of 13 patients with infectious mononucleosis-like syndromes and believed he had found the agent responsible for mononucleosis. However, his suggestion was not widely accepted, particularly in his native Denmark. Yet reports of similar isolations trickled into the literature with tantalizing frequency (73, 136, 235); actually, so much so, that both Blakiston's and Dorland's medical dictionaries still erroneousy give *L. monocytogenes* as the cause of infectious

mononucleosis, without mentioning its role in other disorders! But the number of cases from which the bacterium could not be isolated always continued to be far greater. Finally, with the exception of a small group in the Soviet Union (93, 160), most investigators concluded that there was no real connection between *L. monocytogenes* and infectious mononucleosis. Researchers in Scandinavia even claimed that listeric infection was nonexistent in the human population of their countries. Nyfeldt, it seems, really had not made a very significant contribution after all.

As gathering observations exposed the pattern and meaning of a complicated mosaic, so the middle 1950's gradually transformed the dull, dreary picture of discouragement and disappointment into one of rewarding achievement as the light of new findings revealed and accented the subtle implications and significance of Nyfeldt's early isolations. It showed low-grade listeric infections, not necessarily infectious mononucleosis-like, but rather more flu-like, to be fairly common (89, 93, 94, 136, 235, 271). They were of no serious consequence in nonpregnant individuals, but in pregnant women would almost invariably terminate in grave infection of the fetus if not recognized early. And these infections occurred not only in Middle Europe and America but in the Scandinavian countries, including Denmark. Listeric meningoencephalitis was also uncovered there, and the next few years were to witness the recording of more than 75 confirmed cases of listeric infection in Sweden and Denmark (89, 137, 145, 178) and a few in Norway. Of course, *L. monocytogenes* had always been there, just as Nyfeldt had tried to show, but it, too, was given a different name—unidentified corynebacterium or sometimes diphtheroid bacillus (235).

The first isolation of *L. monocytogenes* from man had another ironic aspect. The cultures that Nyfeldt (174) isolated were all type 3, a type so rare that it was not found again until 25 years later and, as far as is known, has been isolated only eight times since—three times each in Europe and in the USA, and once in Canada and Israel.

After 1929, *L. monocytogenes* was isolated sporadically from man, and interest in the disease followed a rather cyclic pattern. During the war years, few new cases were recorded, and, by 1945, 20 cases of listeric infection had dribbled into the world's medical literature. During the reconstruction of dead, burned-out cities, some 100 cases of listeric infection had been uncovered. Perhaps it is more than mere coincidence that history must record that the real interest in the disease was born in the obstetrical clinic of a bombed-out hospital in Halle.

The story has been told many times in the scientific journals in the way that scientists tell their stories—cold, crisp, concise recitation of established facts: "However, in 1952 Seeliger (235) suggested that the numerous cases of so-called 'granulomatosis infantiseptica' observed by Reiss et al. (215) might be due to *L. monocytogenes* rather than a new species of bacterium, *Corynebacterium infantiseptica*, as reported by Potel (210). During the next few years a series of studies confirmed . . ." But over a friendly glass of cool wine in a cozy cafe in West Berlin it was told like this—

It was 1951. Halle, lying deep in East Germany, had been badly bombed and slow to reconstruct. Life, or even existence, was difficult. Home for many was a shelter of debris against a silent, still-standing wall. Food was poor, meager, and rationed, and essentials, such as milk for pregnant women, were found only in the black markets. The once proud Martin Luther University stood gaunt and naked before a gray German sky, an empty symbol of Halle's former fame, its medical clinics crowded, its staff depleted. Distress, disease, and death were constant companions in the rubble-strewn streets. And among the many who died were the yet unborn. Some of these stillborn infants showed characteristic, distinctive focal necrosis throughout their tiny bodies. Reiss and his co-workers at the postmortem table called it "granulomatosis infantiseptica" for want of a better name. And when the lesion was not seen grossly, in some infants it could be found microscopically. Areas of focal necrosis infiltrated with mononuclear cells. If tissue sections were properly stained, short, slender, rod-shaped bacteria could be seen in and around these necrotic areas. Indeed, the bacteriologist, Potel, had isolated them and had many in pure culture, but could not identify them. There was no well-organized library or source of reference in Halle now; neither could he know that just at this same time the same bacterium was seen for the first time in West Germany in the same type of disease—only in an adult. But Seeliger in Bonn had access to books, received many of the current medical publications, had free contact and communication with Western bacteriologists. Potel could turn only to the Veterinary Investigation Laboratory in Halle. Many times, tired from long, discouraging days in a busy but clumsy and poorly equipped laboratory, he hurried through the darkness to his veterinarian friend, Hartwigk.

There in the drafty, dimly lighted shell of what once had been a laboratory, together they examined cultures, smears, and tissue sections. There was no electricity except for the microscope lamp, no heat, and the tattered, tan paper stretched across openings that once had been windows looking out on Halle's meandering medieval streets rustled restlessly in the night winds. Hartwigk could only say, "I know it isn't *Erysipelothrix*!"

It is easy now to look back across the decade that separates today from that scene and to say, "But they should have known!" Yet, now 10 years later, with 10 years' accumulation of case reports and additional literature, some still fall into the same trap, or escape by calling it an "unidentified diphtheroid."

In addition to the importance of these early studies, they stimulated an awareness of listeric infections in man, particularly in both sectors of Germany (136, 235, 271) and Czechoslovakia (157, 196). Gradually, this awareness spread throughout most of Europe and North America, and by 1956 interest in the disease reached a peak. In 1957 Seeliger and Cherry (236*b*) published their monograph on human listeriosis, its nature and diagnosis. Since that time, interest has remained at a relatively high level—a level which, nevertheless, is still low compared with most other pathogens. Early in 1960, new impetus came as a result of the reports by Rabinovitz et al. (213) and Rappaport et al. (214), who isolated *L. monocytogenes* from the cervical fluid of a number of women in Israel with histories of habitual abortion. This incident confirmed a suspicion advanced by Seeliger (222, 235, 236) as early as 1956. It also stimulated a number of investigations directed toward confirming the validity of the finding.

The true incidence of listeric infection in man remains undetermined. It is striking, and no doubt more than mere coincidence, that of the known number of confirmed cases, well over 1,000 throughout the world, with 400 plus in the United States, more than four-fifths were recorded during the past 5 years. It strongly suggests that the apparent incidence is in direct proportion to awareness of the infection.

During 1959, the Massachusetts Department of Health found that only 55% of 71 laboratories correctly identified *L. monocytogenes*, while four other bacteria known to produce meningitis in man were identified correctly by from 59 to 84% of the laboratories (72). This situation emphasized that an educational program was required if the true incidence of listeric infection was to be determined. The program must be directed toward dispelling three important misconceptions: (i) all small, gram-positive, diphtheroid-like rods isolated from blood, spinal fluid, ear and throat swabs, vaginal swabs, urine, and amniotic fluid are "contaminants" (many so-called "contaminants" and unidentified corynebacteria proved to be *L. monocytogenes* on re-examination); (ii) *L. monocytogenes* attacks only the central nervous system (the bacterium has been incriminated in a variety of disorders as shown in Table 2); (iii) since *L. monocytogenes* grows well and can be cultivated easily after initial isolation, it is always easy to isolate the bacterium from infected material (this statement is not true as indicated in the discussion of METHODS FOR ISOLATION FROM INFECTED MATERIAL).

TABLE 2. *Disorders with which Listeria monocytogenes has been associated*

Meningo-encephalitis—most common in neonates and above 40 years
Flu-like low-grade septicemia in gravida—premature or nonviable termination
Septicemia in perinatal period—usually in prematures
Infectious mononucleosis-like syndrome
Septicemia in adults—often superimposed on other disorders: otitis media, pharyngitis, tonsillitis, sinusitis
Pneumonia
Endocarditis
Localized abscesses—external or internal
Papular or pustular cutaneous lesions
Conjunctivitis
Urethritis
Habitual abortion—confirmed but needs further study
Mental retardation, especially children*
Psychosis in adults*

* Serological evidence only.

Such an educational program also should emphasize that some forms of listeric infection may mimic disorders usually associated with *Mycobacterium tuberculosis*; in stained smears of fluids and tissues, it may resemble *Corynebacterium diphtheriae*, or if poorly stained, *Haemophilus influenzae*. Where such a program is put into effect, listeric infections often cease to be rare. This is shown rather dramatically by the fact that New York has recorded 56 cases of the disease, Louisiana 54, and California 42 (89). Hood (104) at Charity Hospital in New Orleans has now encountered 47 cases (89). This is by far the greatest number by a single institution in this country. Welshimer (280, 281) at the Medical College of Virginia at Richmond is runner-up

with 15 cases (89). In Europe, where the medical profession first became earnestly aware of listeric infections, Potel (223) observed 85 cases at Halle; Träub and Böse (89, 223), 60 cases in Dusseldorf; Patocka et al. (196, 259) in Prague, 53 cases; Jessen (89, 137) in Copenhagen, 22 cases; Winbald (89, 145) in Malmö, 38 cases; and between January and November, 1960, Seeliger (89) received more than 150 cultures isolated from man in Germany. Numerous other instances could be cited emphasizing a relationship between awareness and incidence of listeric infection.

In the USA, where a real interest is merely an awakening, barely audible rustle, 539 cases have been confirmed during 30 years since 1933 when Burn (32) reported the first isolation from a newborn infant. The distribution of cases during this period reveals an illuminating trend: the first 20 years, 40 cases; the next 5 years, 144 cases; and the past 5 years, 355 cases.

It is generally agreed that the increased number of recognized cases reflects a combination of greater awareness and improved methods for isolating the bacterium rather than a real increase in the incidence.

Listeric infection may be manifested by several distinctly different syndromes or associations with disorders of other etiology. This complexity tempted some authors, notably Krepler and Flamm (136), to classify the various disorders according to supposed pathogenesis. However, since so little is known regarding pathogenesis, such a classification is based on mere conjecture. With knowledge presently available, the only classification with any real foundation is that based simply on signs, and it is on this simple foundation that the disorders will be presented here.

It is difficult to determine which disorder is most prevalent. Apparently little effort has been exerted to maintain an accurate tabulation. Historically, meningitis is considered to be the most common form. During recent years, this concept has been contested by several investigators, notably Krepler and Flamm (136), Patocka (196), Potel (223), and Seeliger (235), who claim that septic perinatal infections comprise the majority of cases both in Europe and in the world. It may be true in certain closely limited areas, but is questionable in the overall picture. Gray (89) studied more than 420 cases of the disease from the United States and found perinatal cases to comprise less than 7% of those reported while meningitis in neonates and adults accounted for over 80% of the total. Since recent evidence indicates that inapparent or low-grade undetected infections occur more frequently than is suspected, it may be that they are actually the most prevalent form. Such speculation can be clarified only when the clinician and medical bacteriologist have become sufficiently aware of the manifestations that they no longer pass undetected or masquerade deceptively as a sore throat, flu, or infectious mononucleosis complex.

Before commencing more or less detailed recitation of the several disorders with which the bacterium has been associated, and a few in which it has been accused but never convicted, one fact should be heavily underscored, namely, that *L. monocytogenes* is an expert at charades, pantomiming perfectly many disorders, but showing little originality or ingenuity in producing distinctive disease processes. The single exception which makes the statement valid is perinatal infection. Here, the bacterium can produce a distinctive disease but only in the unborn.

Meningitis or Meningoencephalitis

When *L. monocytogenes* attacks the central nervous system of man, meningitis or meningoencephalitis is the usual result. Meningitis is the primary lesion, and encephalitis, if present, results from extension of the process to the brain. The converse is seen in ruminants where the primary lesion is encephalitis with or without involvement to the meninges. The factors which dictate this difference are not known.

There are no characteristics which distinguish listeric meningitis from that due to other causes. It may affect both sexes and shows no preference for the female as has sometimes been intimated. On the contrary, males are affected far more frequently—191 versus 129 (89). Although all ages may be affected, the first 3 weeks of life and the years beyond 40 are most vulnerable. These two groups comprise almost 90% of all cases. Primary listeric meningitis in children, adolescents, and young adults is comparatively rare and is often superimposed on other disorders. Since meningitis in neonates is actually a perinatal infection, it is discussed in detail under that section, and the following discourse applies only to children and adults.

Although epidemiology and pathogenesis are discussed in their respective sections, a few facts deserve special mention at this point. Listeric meningitis is most frequently observed in patients from low-income groups. It has an extremely high mortality (100%) among alcoholics (89). In older patients, it is sometimes superimposed on neoplastic diseases, diabetes, or other grave illness, and should be suspected in any patients showing meningeal signs. It may occur following

treatment with cortisone or its derivatives, and prognosis is extremely poor in these patients. Guideposts of these maladies may alert the clinician to the possibility of listeric infection and enable him to reach a more rapid diagnosis. Diagnosis, plus prompt and adequate treatment, are essential if the patient is to survive.

There is considerable controversy regarding actual mode of infection. Some evidence supports both those who hold to a hematogenous mode and those who believe in an upper respiratory tract mode.

Onset is usually sudden. Symptoms include severe headache, dizziness, drowsiness, stupor, stiff neck or back, general ill feeling accompanied by vomiting, strabismus, incoordination, and any other symptoms related to disturbances of the central nervous system. Coma often follows shortly. Reflexes may be normal, increased, or decreased. Kernig's and Brudzinski's signs are positive. In addition, some patients may show conjunctivitis, pharyngitis, rhinitis, sinusitis, otitis media, mastoiditis, pneumonia, bronchiolitis, or splenomegaly. The significance of these symptoms is undetermined. They may result either from early low-grade infection with *L. monocytogenes*, betraying the bacterium's portal of entry, or from other causes which may enhance the host's susceptibility to *L. monocytogenes*. A great deal of further study employing more refined isolation techniques will be required before definite answers can be given. Sometimes, particularly in older patients, there is almost immediate loss of consciousness without prior symptoms.

The blood has an increase in polymorphonuclear leukocytes. Monocytosis, often rumored to be present, is actually rare, and should not be looked for as an indication of listeric meningitis.

The spinal fluid may be clear or cloudy and under normal or increased pressure. It may contain large numbers of neutrophiles, especially in the early stages while, later, lymphocytes may predominate. Protein reaction of the fluid is strongly positive and sugar content is lowered. Stained smears often show large numbers of gram-positive rods, either intra- or extracellularly located. This finding, coupled with an increased number of monocytes in the fluid, is almost indicative of listeric meningitis.

L. monocytogenes can be isolated most easily from the spinal fluid. In some cases, it may also be recovered from the blood, especially in the early stages and when meningitis is combined with septicemia. Some hold that listeric meningitis is always septic, but there is ample evidence that this is not true. Portal of entry may be the determining factor. Infrequently, the bacterium may be recovered from the spinal fluid or blood for several weeks or even 3 months after clinical recovery.

The clinical course, as in any bacterial meningitis, is usually acute and stormy. Without therapeutic intervention, death may follow within 24 to 72 hr after appearance of first symptoms. Longer survival, sometimes extending to 1 week or more has been reported, but is rare. In patients that recover, convalescence is slow and prolonged and may require several weeks or more than 1 month. Prompt and proper therapy often shortens the course to less than 2 weeks. Infants appear to respond to antibiotics more rapidly than adults, and some recover in less than 10 days.

Previous to the development of antibiotics, mortality was very high, to 70% or more, and the few who survived were often left with permanent brain damage. In recent years, prompt and adequate treatment with tetracycline antibiotics has resulted in more favorable prognoses. Overall mortality is roughly 50%. However, this figure is somewhat deceiving, since mortality is still extremely high during the first few days of life and beyond the sixth decade, distorting the true picture. During the intervening period, mortality is not over 30%. Although in vivo tests would often indicate the contrary, treatment with penicillin, particularly when combined with one of the sulfa drugs, appears to be satisfactory. Nevertheless, the broad-spectrum tetracycline antibiotics are recommended for all types of listeric infection. It cannot be overemphasized that treatment must be prompt, adequate, and prolonged. Relapses have occurred when treatment was discontinued too soon. Without exception these cases terminated fatally.

Although residual brain damage, hydrocephalis, visual, oral, or auditory defects, and incoordination may be rather frequent sequelae of listeric meningitis in infants and small children, it is rather uncommon in adults.

Lesions seen at necropsy of uncomplicated cases are confined to the meninges and brain. These may be hyperemic and sometimes studded with small grayish-white foci or necrosis. Collections of purulent exudate may be seen particularly around the base of the brain. Occasionally degenerative changes are found in the liver, adrenals, spleen, and kidneys. Pneumonia in the early stages has been reported. Histologically, lesions consist of areas of necrosis infiltrated with neutrophils and some mononuclear cells. They are present in both the white and gray matter. So-called perivascular cuffs

consisting predominantly of mononuclear cells surround many blood vessels. The bacterium may be seen in the necrotic lesions but not in the cuffs.

One of the most disconcerting and confusing features of listeric meningitis is the failure of many patients, even those with severe prolonged cases, to develop detectable antibodies against *L. monocytogenes*. There is no explanation for this. However, it may indicate that since the infection is confined to the central nervous system, the septicemic stage is of such short duration or of such low intensity there is little opportunity for antibodies to develop. It is well known that large doses of the bacterium are required to produce detectable antibodies in laboratory animals. In view of this problem, it is difficult to justify or reconcile many diagnoses of listeric meningitis based only on serological evidence.

It has been suggested that pregnancy is a predisposing factor to listeric infection. If, indeed, this is a true statement, it is definitely not reflected by meningitis Only four or five cases in pregnant subjects have been recorded. In most, the mother recovered and gave birth to a healthy infant. One infant developed meningitis 9 days after birth. One mother died of what appeared to be acute meningitis a few days postpartum. She had been treated with antibiotics for a conjunctivitis and rhinitis during the last month of pregnancy, and all cultures remained sterile. However, the child developed a confirmed listeric meningitis when 10 days old. These few cases among the several hundred reported throughout the world contradict that pregnancy is a predisposing factor to listeric infection. What is true is that if low-grade infections, which may be more common than generally suspected, occur in pregnant individuals, the uterine contents may be infected without serious illness of the mother. There is no evidence that these infections occur more frequently in the pregnant than in the nonpregnant subject. They are merely manifested more often. Present evidence does not support the suggestion that pregnancy predisposes to listeric meningitis.

Encephalitis

Although encephalitis is the most common manifestation of listeric infection in domesticated ruminants, it was unreported in man until 1957. That year, Eck (56) published reports on 10 cases, all of which occurred in the area of Leipzig, Germany. Symptoms were those usually associated with encephalitis—sudden severe headache, high fever, stiffness, and vomiting. These symptoms were preceded by a flu-like syndrome about 10 days prior to the acute stage. Death followed in 2 to 3 days. These cases were so acute that therapy was of no avail. At necropsy, the brains were studded with pinpoint foci of necrosis. Microscopically, there was perivascular cuffing with edema, and some blood vessels were swollen and necrotic. There appeared to be infiltrations of leukocytes from these vessels into the brain parenchyma; in some areas, there were foci of advanced necrosis. These lesions extended into the ventricles and into the meninges. There were no lesions of the choroid plexus. It is possible that listeric encephalitis occurs more frequently than actually diagnosed. Because of its extremely acute nature, an infectious cause is not suspected.

Psychosis

This discussion is presented merely because "listeric" psychosis has been reported (105, 251, 263) and, hence, cannot be ignored. However, the cases of psychological disorders ranging over a period of several months to more than 1 year have been diagnosed only on serological evidence The patients showed intermittent low fever, bizarre hallucinations, manic-depressive complex, delusions and illusions, altered reflexes, and the whole gamut of disorders that could be associated with the psychology of abnormal people. They showed few symptoms indicative of active or even chronic, low-grade infections. None had marked fluctuations in titer against *L. monocytogenes*, yet the bacterium has never been isolated from any of the individuals in question. To avoid being inconsistent with what has been stated under *Serological Diagnosis of Listeric Infection*, no further comment is necessary.

Perinatal Infection

Perinatal listeric infection, as pointed out in the introduction of this section, had an interesting, although somewhat confused history. Had it not been for the coincidence that Seeliger (235) encountered a case of listeric septicemia in an adult in 1950, the true role of *L. monocytogenes* in the perinatal period may not have been established as early as it was. Although some of the first isolations of the bacterium were made from neonates [(32); the second isolation made by Murray et al. (164) was from a pregnant rabbit], it seemed as if the bacterium deliberately evaded being incriminated in its attack on the unborn. Somehow, during the period from 1933 to the early 1950's, the emphasis was always diverted to the bacterium as a cause of meningitis in adults. Yet, careful paging through the published case material would have shown that the suspi-

cion was not at all justified. Actually the number of isolations from neonates and young children was slightly greater than that from adults.

The importance of perinatal listeric infection as a cause of death or permanent mental or physical impairment in the newly born remains to be determined, but the rapidly accumulating published reports suggest that it may be of greater significance than previously suspected. Seeliger (235) and Alex (1) feel that, following erythroblastosis fetalis, syphilis, and toxoplasmosis, *L. monocytogenes* is one of the most frequent causes of fetal damage or infant death. However, with present methods of reporting disease, it is difficult to determine. In hospitals where such an effort has been made, the incidence ranges from 0.15% of the total perinatal mortality, according to Breuning and Fritzsche (30), at the University Women's Clinic at Leipzig, Germany, to 2.0% at the University Women's Clinic at Halle (1). At certain times in some areas, it has been much higher than this. Occasionally, mortality has almost reached epidemic proportions for short periods. Sometimes there are several cases in rapid succession; then the infection disappears, and a year or more may pass before it is seen again. No epidemiological links can be established in these instances, and there is no explanation for the periodic fluctuation.

Also undetermined is the effect of infection on the mother's reproductive capability. In most instances, there appears to be no long lasting damage to the reproductive system. However, there is substantial evidence that chronic, low-grade infections may be responsible for so-called habitual abortion as indicated under that section.

Since all so-called scientific classification is merely man's feeble effort to create, artificially and arbitrarily, some order in nature, the mother's role in perinatal listeric infection should not be denied definition. However, because she plays such a passive, unwitting, and unwanted role, classifiers seem to have ignored its desirability completely, and it remains, as the raven in Poe's poem, nameless. Perhaps a good name would be simply "low-grade listeric septicemia during pregnancy."

Abortion or perinatal septicemia. A broad, general clinical picture of perinatal infections due to *L. monocytogenes* drawn from case histories in the USA and Europe has recently been published by Gray, Seeliger, and Potel (91*a*). Listeric abortion has occurred as early as the second gestation month (89), and there are several suggestions based on both cultural and serological studies that the embryo may be infected at an even earlier stage. Studies of artificially infected rabbits strongly suggest that the fetuses are not aborted until the latter part of gestation, even though the dam is exposed continually throughout the entire gestation period (84).

There are no clinical signs which distinguish listeric infection from several other common disorders of pregnancy. In most instances, the mother is not seriously ill. It appears to be primarily an infection of the uterine contents. Some mothers can recall nothing that would suggest infection. Many others recall "flu-like" episodes or symptoms suggestive of pyelitis or cystitis a few weeks or a few days prior to parturition. These symptoms appear to result from low-grade listeric bacteremia. Blood cultures taken at the time often reveal the bacterium, and on at least one occasion it was isolated from the tonsils (132). If appropriate treatment with tetracycline antibiotics is initiated promptly, most women give birth to normal infants. However, these infants should be watched closely during the first 6 months for early signs of meningitis.

If not treated, there is a marked decrease in fetal activity shortly after these symptoms disappear. Of 85 patients observed by Potel (223), only one stated that fetal movements were normal throughout the entire gestation.

A composite picture drawn from accumulated case histories appears as follows. Some time during the last half of pregnancy there were sudden chills accompanied by rise in temperature, sore throat, headache, mild dizziness, pains of the lower back, discolored urine, and perhaps diarrhea. These symptoms persisted for several days, and then disappeared. Following short periods of well-being the symptoms did or did not reappear. Shortly after the first attack, fetal movements became weaker and less frequent. Premature delivery of stillborn or acutely ill infants followed in a few days. The amniotic fluid was discolored or murky but had no abnormal odor. Sometimes small gray necrotic foci could be observed in the placenta. After parturition the mother was asymptomatic, and the postpartum course was without further complication.

In spite of the absence of symptoms in the postpartum period, *L. monocytogenes* can be isolated from the vagina, cervix, or urine of the mother for periods ranging from a few days to several weeks, occasionally 1 month or more. Cultures prepared later than 10 days often failed to reveal significant bacterial growth. Mencikova (157) cultured menstrual blood from women who had given birth to infected infants but could

not isolate the bacterium from them. It appears that the bacterium was shed rather quickly by the mother and that its presence in the uterus in the immediate postpartum period was without detrimental effect. The metritis, which, based on experimental findings in animals (84, 95, 159, 198), almost certainly existed in all cases, was not serious, and the uterus usually returned to normal function in about the same length of time as after uncomplicated pregnancies.

In contrast to the mild involvement of the mother, the infant was either stillborn or born acutely ill, especially if birth was premature. If born alive, the infant often expired within a few minutes or hours. Reports of survival of more than 3 days are rare, but some have lived as long as 2 weeks.

Ante-mortem diagnosis is difficult to establish since there are no pathognomonic symptoms; those which occur may mimic the signs of a number of other disorders of the newborn. The usual symptoms reflect disturbances of the respiratory, circulatory, or central nervous system: dyspnea, lowered body temperature, cyanosis, thirst, vomiting, spasms, soft whimpering, early passage of meconium and mucuslike stools. Diarrhea may occur, and occasionally red rash or papules containing the bacterium may be found over part or all of the body. Sometimes, there is a purulent discharge from the eyes and the bacterium may be isolated from this source. Often the body is in a state of hyperextension and convulsions may be observed. If the infant survives for several days, suppurative meningitis often develops which usually terminates fatally or may lead to hydrocephalis or permanent mental deficiency if the infant survives.

If listeric infection is suspected, smears and cultures of the meconium should be made immediately. The bacterium can usually be found in large numbers in the material (2). However, as indicated earlier, it may not always grow readily from this source (277). It has also been suggested that roentgenograms of the chest may reveal lesions in the lungs. In any event, it is imperative that treatment with massive doses of tetracycline antibiotics be initiated without delay.

The lesion seen most frequently at necropsy is focal hepatic necrosis. This led Reiss et al. (215) to name the disorder granulomatosis infantaseptica before the true etiology was known. The term is still sometimes applied, but has gradually been replaced by the more exact name perinatal listeric septicemia. The foci are grayish-white, often slightly raised, pinpoint to 1.5 mm in diameter, and may range from scattered to almost complete studding of the organ. The adjacent hepatic parenchyma may appear normal or may be yellowish, swollen, and necrotic. Similar foci are often seen in the adrenals, spleen, lymph nodes, lungs, and brain. Less frequently, they are observed in the heart, oral mucous membrane, tongue, tonsils, esophagus, stomach, and small and large intestine, including the rectum. Hydroperitoneum, peritonitis and sero-fibrinous exudate on the visceral organs, particularly the liver, may be seen. Complete absence of gross lesions is rare, but when it occurs lesions may be found histologically.

In general, the lesions seen in all organs are characterized by well-defined areas of focal necrosis and infiltration with polymorphonuclear and mononuclear cells without alteration of the adjacent tissue. Often, these foci appear to be associated with blood vessels, but the significance of this observation remains undetermined. Numerous bacteria may be seen in and around these lesions. Most authors call attention to the toxic nature of this reaction. Many German pathologists mentioned a second type of lesion which was found most commonly in the liver. It consisted of an increase in mesenchymal cells resulting in an increase in reticular cells, histiocytes, monocytes, and epithelioid cells. The lesion was referred to as "granuloma" and appeared to be an older lesion; *L. monocytogenes* has been seldom seen in or isolated from it. Simon (244) described similar lesions in meninges of neonates and called them "listerioma." This granuloma-type lesion leads to much of the confusion between infections due to *L. monocytogenes* and those due to *Mycobacterium tuberculosis*. It could be interjected here that most German pathologists who studied lesions in neonates considered them to result from endothelial cell proliferation. This is in exact contrast to Bloom's (25, 26) view; he stated that there was no true evidence for the stated opinion.

Bronchopneumonia, due to *L. monocytogenes* and usually described as "atypical pneumonia," is infrequently seen in newborns. It has been observed both in Europe and this country. Mencikova (157) reported that often the true cause could be determined only by refrigeration of lung tissue. It is not known how often infection of this site occurs, and it is possible that some cases are overlooked.

Twins born to infected mothers are usually both infected. Occasionally one is born apparently well and develops no indication of illness. Rarely, the uninfected twin develops the disease somewhat later during the neonatal period. There is no explanation for this variation, but it has been

observed also in artificially infected animals (84). Although conjecture, it is possible that identical twins are always equally infected while fraternal twins may not necessarily be. The exact nature of the variation has not been investigated successfully.

Several rather elaborate schemes have been devised to map the bacterium's possible passage from mother to fetus. However, the true route remains known only to the bacterium and it has not yet divulged its pathway to the few who have attempted to trace its journey. True, it may go by the complicated route: from mother's blood stream during low-grade septicemic stage to maternal placenta to fetal placenta, then through the navel vein, producing fetal septicemia. As a result, the bacterium could be passed with the fetal urine into the amniotic fluid which is aspirated or ingested by the fetus, thus giving rise to the widespread respiratory tract or gastrointestinal tube alterations seen at necropsy. *L. monocytogenes* can readily produce massive septicemia, especially in the very young, without being aspirated or ingested directly. However, if exposure were very light, septicemia might not result, but rather low-grade infection could result with pathogenesis as described in the following paragraphs. Few hold the view advanced by Krepler and Flamm (136, 223) that the mother is reinfected from the developing infection in the fetus, and that this reinfection, and the changes produced by it in the placenta, are the actual cause of abortion or premature delivery of the fetus.

Meningitis. Some infants born apparently well develop listeric meningitis a few days to 3 weeks postpartum. These cases comprise almost 40% of all human listeric meningitis reported in the USA (89). There is strong evidence that infants infected prenatally or during birth are a result of inapparent infection of the mother, rather than of environmental contact with the bacterium during the neonatal period. Studies on artificially infected pregnant rabbits substantiate this route of infection (84, 164). As in perinatal septicemia, the mother can often recall a flu-like syndrome shortly before parturition. In a few instances, the bacterium has been isolated from the mother's blood at this stage and the infants, apparently well at birth, developed meningitis during the neonatal period (89, 104). It may be possible that many individuals either harbor the bacterium or experience low-grade infection which, when it occurs in the nongravid subject, is of no serious consequence, but in the gravid subject invariably terminates in infection of the fetus. The enhanced susceptibility of embryonic tissue to insult by *L. monocytogenes* is amply documented (84). Focal necrotic lesions, similar to those seen in perinatal septicemia, that have been found in some infants which expire is further support for the idea that these infants were exposed prenatally.

At the onset, there is loss of appetite, excessive crying, extreme irritability, coughing, running nose, and high fever. These symptoms worsen rapidly and are followed by convulsions or coma, or both. There are no distinguishing symptoms or clinical features; however, laboratory findings of the gram-positive bacilli and occasional increases in number of mononuclear cells in the spinal fluid may differentiate it from other bacterial meningitides. Some infants may have slightly elevated monocyte counts. If treated early, the infant usually responds well to tetracycline antibiotics (89, 104).

If infants are actually infected prenatally, as suggested, one may consider the fact that in the USA neonatal meningitis has comprised more than 40% of all cases whereas perinatal septicemia represented only 7% of the total. In contrast, in Europe, especially in Germany and Czechoslovakia, perinatal septicemia has accounted for almost 75% of the known cases. This could be interpreted as an indication that European mothers are, in general, more heavily infected than those in this country. This postulation can be supported experimentally. Young rabbits born to heavily infected does die with septicemia while those born to lightly infected does most often die of meningitis (84, 159). Extrapolation of these data may possibly explain the difference in incidence of the two forms of listeriosis on the two continents.

It is not known what effect perinatal infections may have on later life. Hydrocephalus is a common sequel to meningitis in neonates, and many expire at an early age or are mentally retarded (89, 136, 235). There is also considerable evidence, based on both naturally and artificially induced infections in young animals, that vegetations may develop on the heart valves. This lesion has been reported a number of times in animals, but is seldom mentioned in reports dealing with human neonates.

Mental retardation in children. Since it is established that recovery from listeric meningitis or encephalitis in man and animals may result in permanent brain damage, it has been suggested that some inapparent perinatal infections may lead to retarded mental development during childhood. Based on studies by Lang (144) which documented that among a group of mentally retarded children in which the etiology was not

established, 43 of 87 had significant titers against *L. monocytogenes* while only 5 of 50 children in which the etiology was established had similar titers. Miller (89) could not confirm this work in a study of more than 200 sera from mentally retarded children in New York State. If similar studies have been conducted elsewhere, they have not been reported. With the known inconsistent and unpredictable results often obtained in serological reactions with *L. monocytogenes*, as exposed under *Serological Diagnosis of Listeric Infection*, no definite conclusion can be reached. Nevertheless, it is well within the realm of possibility that inapparent listeric infections early in life may account for a surprising number of mental deficiencies.

Effect on reproductive system. Today there is much speculation that *L. monocytogenes* may be the cause of chronic infection of the female genital tract. However, there is considerable contrary evidence, based on both naturally and artificially induced infections. Potel and Alex (211) studied 19 women through 26 subsequent pregnancies after they had given birth to infected infants. All but three terminated in birth of an apparently normal, healthy, full-term infant. *L. monocytogenes* could not be incriminated in the three nonviable pregnancies. Similar results have been the experience of most other investigators. Also, it is well established that most animals which give birth to young infected with *L. monocytogenes* conceive normally and produce healthy young after rebreeding (85). A mother who gave birth to an infected infant need not necessarily fear a subsequent pregnancy. However, she and her obstetrician should be well aware of the potential hazards. Blood for culture and serological studies and cultures from the genital tract should be made at frequent intervals. The laboratory staff should be well aware of the possible difficulties of isolating the bacterium under these circumstances. Appropriate therapy, tetracycline antibiotics, should be initiated immediately if there is sudden rise in titer, or marked change in the blood constituents, or if cultures reveal the bacterium, or if, even in their absence, the patient develops flu-like symptoms. With proper precautions, such pregnancy, in almost all instances, should terminate successfully. Regardless of the condition of the infant at birth, it should be observed closely for at least the first year for any sign suggestive of early meningitis.

Possible role in habitual abortion. The idea that *L. monocytogenes* might be associated with cases of so-called "habitual abortion" is not new. As early as 1955, Gray et al. (84) produced such a sequence in rabbits after single ocular exposure of pregnant does. One doe aborted during each of three consecutive gestations, and the bacterium was isolated from the aborted conceptus and vagina of the doe after each abortion. About this time, Seeliger (235) observed that approximately 16% of the women who had given birth to infants infected with *L. monocytogenes* had either aborted or given birth to stillborn infants during previous pregnancies. Also, Rost et al. (224) found sharp rises in antibody titer against *L. monocytogenes* at the time pregnancy was interrupted in a number of women with histories of habitual abortion. When these women were treated with a combination of tetracycline antibiotics and sulfa drugs throughout pregnancy, many gave birth to apparently normal, full-term, healthy infants. Similar observations were made subsequently in other parts of Germany and Czechoslovakia (223, 235). Unfortunately, the role of *L. monocytogenes* in these cases could not be confirmed by isolation of the bacterium from either the aborted conceptus or the mother, and diagnosis of listeric infection was based entirely on serological findings.

Recently, Rabinovitz et al. (213) and Rappaport et al. (214) at Hadassah Municipal Hospital, Tel Aviv, Israel, reported the isolation of *L. monocytogenes* from the cervical secretion of 25 of 34 women with histories of repeated abortion. Nineteen infected women were treated with large doses of penicillin and sulphamethoxypyridazine (Lederkyn). Of these, 10 gave birth to healthy infants and 4 were in normal course of pregnancy (89). *L. monocytogenes* could not be isolated from 87 other women with no history of repeated abortion.

These publications constitute the first documented, actual association of *L. monocytogenes* with habitual abortion in women. More recently, Oehlschlager (176) presented additional support for this concept. His patient suffered two miscarriages followed by premature delivery of an infant that died 53 hr after birth with confirmed listeric septicemia. Four months later the patient was again pregnant and still harbored *L. monocytogenes* in her vagina. She was treated with oxytetracycline vaginal suppositories and pHiso-Hex douches and gave birth to an apparently normal full-term infant.

These publications not only confirm suspicion that has been held for 6 years but also re-emphasize the value of prompt diagnosis and proper therapy. They also have stimulated a number of investigations to strengthen the validity of apparent association of *L. monocytogenes* with habitual abortion. However, there has been in-

sufficient time for significant data to accumulate.

Norys (172) cited a case typical of those that lend intriguing circumstantial support and invite continuing speculation of the role of *L. monocytogenes* in some cases of habitual abortion. Her patient had a history of sterility dating back to 1953. Surgical intervention for salpingitis resulted in conception which terminated in stillbirth in 1956. In mid-1958, she was threatened with abortion during the fourth month of pregnancy. She had a titer against *L. monocytogenes* type 1 O antigen of 1:400 and 1:200 against H antigen. In spite of treatment with sulfa drugs (Supronal) and penicillin, she delivered stillborn identical twins 3 months prematurely. Both showed nonspecific enterocolitis and peritonitis. Although there were no gross or microscopic lesions characteristic of listeric infection and the bacterium could not be isolated, the author attributed fetal damage to low-grade listeric infection during the fourth gestation month. Numerous similar cases could be recited.

It is striking, although not satisfactorily explained, that many mothers who gave birth to either infected infants or those who developed listeric meningitis in the neonatal period have histories of previous nonviable pregnancies. Just to be perverse, it could be argued that women with vague, nonspecific, chronic disorders of the reproductive tract which lead to repeated nonproductive pregnancies are merely more susceptibe to listeric infection and that the bacterium is not actually an instigator at all in these cases but rather an unwitting accomplice. Nevertheless, these observations make it imperative that a conscientious effort be made to confirm or deny this tantalizing suspicion.

Potel (89, 211, 223), who has studied more patients with perinatal listeric infection than any other investigator, has continually and consistently opposed the view that the bacterium may cause repeated abortion. He still holds to the opinion. It will be interesting to see whether he or his colleagues are correct. In the meantime, it would be unwise to draw general conclusions from the reports of habitual abortion due to *L. monocytogenes*. Although Rabinovitz et al. (213) and Rappaport et al. (214) observed a relatively large number of patients, the study was confined to a rather small geographical area where the general distribution of *L. monocytogenes* has received but scant study. It has been isolated from animals in Israel on several occasions (214), and there is a report of a small but almost epidemic-like outbreak among five newborns at the Central Emek Hospital, Afula (149). It was not stated whether these patients with histories of repeated abortions originated from rural or urban areas, or whether they were concentrated within a limited area where listeric infection might be endemic. However, if, for the moment, it is assumed that these data are typical for all areas, they imply that approximately 70% of all women with histories of repeated abortion may suffer from inapparent listeric infection. If further extended to include the 87 controls with no history of repeated abortion and from whom *L. monocytogenes* could not be isolated with the same culture techniques, it would indicate that at least 20% of all women harbor the bacterium in their genital tract. If true, it is almost certain, especially in view of the extreme susceptibility of the uterine contents to listeric infection under exprimental conditions (84, 95, 159, 198), that perinatal listeric infections would be much higher than available information indicates. If the suggestion that a relatively high percentage of children with retarded mental development may have suffered inapparent listeric infection in the prenatal period can be accepted as valid (144), and if most infants who develop listeric meningitis during the first few weeks of life are actually infected prenatally as a result of undetected infection in the mother, it is quite possible that more women harbor the bacterium in their genital tracts, especially during pregnancy, than has been suspected. Although *L. monocytogenes* has been isolated occasionally from the genital tracts of apparently normal animals (85, 214), only two such isolations are known from apparently normal women: one from the cervix (89), the other from urine of a pregnant woman (89). The true picture cannot be revealed until more concentrated effort is made by both the medical profession and the medical bacteriologist to determine what the actual incidence may be.

The large number of case histories which have accumulated during recent years, unfortunately, sheds little light on the possible mode of infection of the mother. Laboratory studies on pregnant animals suggest the oral route to be the most probable portal (84, 95, 159, 198). This concept is also supported by circumstantial evidence from Europe. In many of the early cases reported from East Germany, the mothers were from rural areas and gave histories of drinking raw milk (1, 136, 211, 223, 235). In one instance, Potel (223) isolated *L. monocytogenes* from milk of a cow with atypical mastitis. A woman who drank raw milk from this cow gave birth to stillborn, premature twins and *L. monocytogenes*, of the same serotype as that isolated from the milk, was isolated from each. The report remains the only established epidemiological link actually implicating raw

milk, or any other food, as possible source of infection.

The frequent occurrence of mild respiratory-tract distress shortly before parturition strongly suggests that the mother may be infected by this route.

Since the bacterium may persist for relatively long periods in the genital tract, it could constitute a source of infection for the next pregnancy. It also suggests the possibility of a venereal mode of transmission, particularly among those indulging in promiscuous relations. This is supported by Wenkeych (282), who isolated *L. monocytogenes* from the urethral exudate of five men with gonorrhea who had shared a common sex partner. In at least one instance, *L. monocytogenes* has been isolated from the semen of a husband whose wife was known to harbor the bacterium in her genital tract (89). If more such specimens from both partners were cultured in a laboratory alert to the possible presence of the bacterium, and with employment of correct isolation techniques, the incidence might be found to be fairly high. However, as in all other forms of listeric infection, there is little information of real epidemiological significance. It is fortunate that *L. monocytogenes* has never been isolated from a stork, or surely this poor bird would be blamed not only for his big bill but also for transmitting the bacterium to newborn infants.

Infectious Mononucleosis

Infectious mononucleosis, or glandular fever as it is sometimes called, was the first disorder in man with which *L. monocytogenes* was associated. Unfortunately, the relationship has not been appreciably clarified since the day in 1929 when Nyfeldt (174) first suggested the association. As mentioned in *Retrospect*, the suggestion was scoffed, lauded, doubted, accepted, almost abandoned, intensively pursued, but never really resolved. True to its characteristics, *L. monocytogenes* either effectively evades the investigator's probe or only grudgingly gives an occasional insight into its ways. In its relationship to infectious mononucleosis, the microbe has been particularly evasive. All efforts to brush the haze aside, sharpen the focus, and produce a clear understanding have ended in frustration. Just when the image appeared to clear, new evidence popped it out of focus again. This frustration has continued for 34 years, and the true story is not yet revealed.

Although Nyfeldt (174) is rightfully credited for first associating *L. monocytogenes* with infectious mononucleosis, Coon and Thewlis in 1922 and Baldridge et al. in 1926 isolated gram-positive rods from lymph nodes of patients with infectious mononucleosis, confirming the words of Solomon- "There is nothing new under the sun." The first description of infectious mononucleosis, a complex characterized by sore throat, intermittent fever, swollen lymph nodes and spleen, high percentage of "peculiar" lymphocytes in the circulating blood, and high titer of sheep erythrocyte agglutinins (heterophile antibody detectable by Paul-Bunnell test) in the serum, is generally ascribed to Pfeiffer in 1889, but, as might be expected, Morozkin and Lebedeva (160) claim that it was first reported by Filatov in 1885. But history does not solve current problems; it only gives a better insight into them. The present task is to clarify the problem.

In 1951, Girard and Murray (73) made a valiant attempt at least to review, if not to solve, this problem. Their effort remains one of the most comprehensive; during the intervening years, little definite information or convincing clues have been added to this tantalizing subject. Girard and Murray concluded, as did Stanley (199) before them, that there may be three forms of infectious mononucleosis: (i) Paul-Bunnell and *L. monocytogenes* agglutination test, both positive; (ii) Paul-Bunnell positive, *L. monocytogenes* negative; and (iii) both tests negative. Group 1 would indicate infection with *L. monocytogenes* while 2 and 3 would indicate viral etiology. The grouping is based on serological evidence and may be an oversimplification of the problem, especially regarding group 3. Many patients with infectious mononucleosis-like complex due to *L. monocytogenes* have failed to develop antibodies against the bacterium. If the bacterium were not isolated from these cases, they would pass undetected in such scheme. Also, Stanley considered a titer of 1:16 against *L. monocytogenes* as significant. With today's knowledge, titers of this value are disregarded, essentially negating the entire scheme.

Superficially, there appear to be at least two causes of infectious mononucleosis: virus(es) and *L. monocytogenes*. The two (or more) agents seem to produce identical clinical pictures, and can be distinguished only if *L. monocytogenes* can be isolated. In the USA, only three cases of mononucleosis associated with *L. monocytogenes* are known (89). In Germany, Urbach and Schabinski (223, 271) reported an outbreak which involved 58 student nurses, and *L. monocytogenes* was isolated from pharyngeal swabs of three. Many of the others had high serum titers—some above 1:800—against *L. monocytogenes* while the Paul-Bunnell test was negative. Perhaps in such an instance, it might be concluded that all were infected with the bacterium. Most other investi-

gators have not had such consistent results. Morozkin and Lebedeva (160) claimed the isolation of *L. monocytogenes* from an undisclosed number of 158 patients with infectious mononucleosis. However, when some of these cultures were later studied by Shlygina (241) not one proved to be the suspected bacterium. It is not known whether this episode has dislodged some of the other Russian investigators (93, 94) from their belief that infectious mononucleosis is "one of the most typical forms of listeriosis" (93).

In a sense, the concept of "listeric infectious mononucleosis" is somewhat of a paradox. The bacterium is usually associated with either acute, grave disease processes or relatively benign, almost inapparent infection, neither of which simulate true infectious mononucleosis. Few have attempted to explain this point; most merely accept it. Krepler and Flamm (136) ventured an intriguing postulation. It is known that a sustained monocytosis enhances the rate and quantity of antibody production (74, 254); if listeric infection occurs simultaneously or is superimposed on infectious mononucleosis of viral origin, antibody production against *L. monocytogenes* should be enhanced, thus preventing serious listeric infection. Although they did not mention it, the increase in antibody might explain the infrequent isolation of the bacterium from such cases. However, the concept is partly based on the unfounded assumption that the patient developed antibody against *L. monocytogenes*. However, it is contradicted by a majority of the cases, as has been repeated an almost monotonous number of times. It is doubtful that antibody production or activity plays a significant role.

A second and justifiable assumption is that primary infection was of viral origin and *L. monocytogenes* just happened to be there. In defense, Krepler and Flamm (136) cite Jorke (117) who, incidentally, gave an excellent review of the subject, and likened the relationship of *L. monocytogenes* to infectious mononucleosis as ". . .wie der Influenza-Bacillus (*B. hämophilus* Pfeiffer) bei der Grippe." It is perhaps the correct explanation, and has been supported to a degree by Urbach and Schabinski (271).

The accumulating evidence that many apparently normal individuals harbor *L. monocytogenes*, and that *L. monocytogenes* is often associated with some other disorder makes the concept even more tenable. It is highly probable that whenever *L. monocytogenes* is associated with infectious mononucleosis it is never the primary agent and plays no significant role in the clinical picture. At present, this theory appears to be the most logical explanation. This concept satisfies all the requirements but one: how to explain the absence of heterophile antibodies in some patients from which *L. monocytogenes* has been isolated. Could it be that in some still unexplained manner the bacterium interferes with heterophile antibody production? As far as is known, the interference has never been investigated, but it might well be worth a try by an inquisitive investigator. Nevertheless, it might merely mean that the patient had an infectious mononucleosis-like disorder but not true infectious mononucleosis.

This concept is further supported by the failure of most patients with confirmed listeric infections to develop circulating monocytosis and the bacterium's inability to produce heterophile antibody. Both of these facts are well documented: the former by survey of published reports (136), the latter by numerous studies showing that absorption of serum with *L. monocytogenes* has no effect on its heterophile antibody titer and absorption with sheep red cells has no effect on *L. monocytogenes* titer (73, 136, 235). Also, in spite of numerous attempts, there is presently no evidence that *L. monocytogenes* alone produces heterophile antibody (73, 136, 235). For these reasons, it seems unlikely that *L. monocytogenes* is capable of producing true infectious mononucleosis.

Further evidence against *L. monocytogenes* as the etiological agent of infectious mononucleosis has been the isolation of the bacterium from not only the blood but also the spinal fluid of patients showing symptoms suggestive of the disease. It strongly implies that the patients were actually experiencing low-grade, early forms of listeric meningitis with associated bacteremia and involvement of some lymphatic tissue, rather than true infectious mononucleosis.

The preponderance of evidence indicates that *L. monocytogenes* may be associated with but never the primary cause of true infectious mononucleosis. Listeric infection may be superimposed on infectious mononucleosis in precisely the same manner that the infection may be superimposed on neoplastic diseases, diabetes, or other disorders with which it has been associated as secondary infection. It is true that *L. monocytogenes* may produce syndromes closely simulating infectious mononucleosis and characterized by fever, sore throat, and enlarged regional lymph nodes with or without involvement of the central nervous system. However, this entity is not to be confused with classical infectious mononucleosis.

Septicemia

The characteristic clinical and pathological history of listeric septicemia of newborn infants has been described under *Perinatal Infection*. In

adults, septicemia is most commonly detected in pregnant subjects in which it may lead to infection of the fetus, or with those with disorders due to other primary causes. It is highly probably that low-grade septicemia, or, more precisely, bacteremia, is more prevalent than generally suspected and may pass undetected since, in nonpregnant subjects, it appears to be of no serious consequence unless complicated by other disorders. It is supported by the isolation of *L. monocytogenes* from a number of clinically well individuals. Uncomplicated cases rarely develop into an acute fatal disease, unless it leads to meningitis.

Uncomplicated primary listeric septicemia in adults is rare, so rare, in fact, that it is difficult to describe. There are no clinical symptoms which distinguish this type of septicemia from that of other etiologies. A diagnosis can be confirmed only by isolation of *L. monocytogenes*. Usually the infection responds favorably to antibiotic treatment and mortality is relatively low. So few cases have come to postmortem that it is almost impossible to present an accurate pathological history. The gross and microscopic lesions simulate those of septicemia in newborns. Focal necrosis is the most characteristic lesion and is usually most pronounced in the liver and spleen. Sometimes such foci are found in the lungs, kidneys, mesenteric lymph nodes, and other viscera. Sometimes there are other lesions: pneumonia, enteritis, or nephritis. It is not known whether they result from listeric septicemia or are distinct secondary disorders of other etiology. However, bronchopneumonia due to *L. monocytogenes* has been reported in infants and adults (89, 157, 235). Although rare, the physician and laboratory personnel should be aware of the possibility of listeric septicemia.

Listeric septicemia superimposed on other disorders is less rare. It is most often associated with neoplastic disease, diabetes, alcoholism, and other debilitating conditions. It often follows administration of cortisone or its derivatives. The clinician should be alert to this hazard whenever cortisone is given, particularly to elderly patients. It is well established that cortisone greatly enhances susceptibility of laboratory animals to listeric infection (85, 170). The same may be expected for man, and listeric infection should be suspected whenever gram-positive rods are isolated from blood of patients on such a regimen. Such cases are often highly acute and prognosis poor. In fatal cases, septicemia is the ultimate cause of death.

It is difficult to determine what effect listeric septicemia has on the course of the primary disorder. Some patients expire quickly while others appear to recover from the infection only to die shortly thereafter due to the primary cause(s) or further complications thereof. Although the patients may have extensive lesions, at postmortem they are seldom those characteristic for listeric septicemia. Considerably more study is necessary before the true role of *L. monocytogenes* in such cases can be determined.

The fact that listeric infection often occurs in patients already debilitated by serious illness lends strong support to the contention that many individuals harbor *L. monocytogenes* and that its presence is manifested only when the body defenses are lowered. Some patients had been disabled or hospitalized for relatively long periods prior to onset of the septicemia, and it seemed unlikely that they would have been exposed to the bacterium by environmental contact. The likelihood cannot be determined definitely until there is better understanding of the epidemiology and pathogenesis of all listeric infections. In the meantime, it can only be stated that listeric septicemia does occur and that it can have serious consequences in pregnant subjects, those suffering from neoplastic or other chronic, potentially terminal disorders, or it may lead to listeric meningitis in apparently healthy individuals. Perhaps predisposing factors are an essential part of all listeric infections.

Other Disorders

L. monocytogenes has also been infrequently associated with a rather diverse variety of clinical entities. Most of these are not particularly serious but should be mentioned, since there is strong evidence that the bacterium is the primary agent and that the patients may develop more serious infection, and, only if there is an awareness of these disorders, can a correct diagnosis be made. Listeric infection should be suspected whenever gram-positive rods are isolated from patients having the following clinical expressions.

Endocarditis. Endocarditis due to *L. monocytogenes* has been reported both in this country and Europe (89, 136, 235). It has no distinctive characteristics to distinguish it from endocarditis due to other causes and can be diagnosed only by isolation of the bacterium. In some instances, *L. monocytogenes* has been isolated from the blood over a relatively long period, and some patients develop significant antibody titers. Most cases respond well to antibiotic treatment and, as far as is known, there have been no serious complications. It is highly probable that the prevalence is much greater than the five presently published cases would suggest. How high might it be if all

the "contaminating diphtheroids" isolated from blood cultures had been correctly identified instead of discarded?

Abscesses. Both external and internal abscesses due to *L. monocytogenes* have been reported (89, 235, 274). Usually, they occur in elderly patients but they have also been seen in adolescents. External abscesses are most frequently associated with the cervical lymph nodes. They are fistulated and contain bloody pus, which in stained smears shows many phagocytes with large numbers of ingested bacteria. The abscess may rupture spontaneously or require excision. Sometimes, *L. monocytogenes* can be isolated in pure culture, but it may be mixed with other bacteria, particularly *M. tuberculosis*. Response to appropriate treatment is favorable, but, in at least one instance, listeric meningitis has been a sequel (235).

Internal abscesses are frequently associated with chronic disabling conditions. Most are detected strictly by chance at exploratory or postmortem examination and their true nature is determined only by culture. They are most frequently found on or in the liver and spleen, occasionally in other viscera, and rarely in the brain where they may give rise to symptoms suggestive of brain tumors.

It is obvious that the true etiology of the abscesses can be determined only if the medical bacteriologist is aware that *L. monocytogenes* may be associated with this condition.

Cutaneous lesions. A papular or pustular cutaneous eruption has been the most recent syndrome to be linked with *L. monocytogenes*. With exception of the report by Novak (173), the other recorded observations are almost identical, although they appeared independently in the Netherlands (51), Germany (120), and the United States (189). Each involved veterinarians who had handled bovine fetuses infected with *L. monocytogenes*. Within 48 to 72 hr after exposure, there was malaise, headache, dizziness, a short period of mild fever, and the appearance on the hands and arms of round, red, tender papules containing *L. monocytogenes*. This change was accompanied by slight swelling of the axillary lymph nodes. After several days, some papules developed white pustular centers. There was rapid favorable response to antibiotic or sulfa drug therapy. In at least two instances (51, 189), the culture isolated from the aborted material was of the same serotype as that from the cutaneous lesions. Serotype was not mentioned in the other report. It is not known how frequently the infection may occur among veterinarians and others who may contact infected material, but pass undetected because of its benign nature, short duration, usual disregard for "pimples," and lack of awareness that it may be listeric infection.

The patient of Novak (173) developed sudden fever, vomiting, and swollen glands of the face, lower jaw, and neck after ultraviolet treatment for chronic inflammation of the ovaries. Shortly, papulous exanthema with bright red eruptions and vesicles developed on the skin in the swollen area, and *L. monocytogenes* was isolated in pure culture from these lesions. The patient had no other symptoms suggestive of listeric infection and made an uneventful recovery.

Although it is well documented that many newborns and also some adults with listeric septicemia may have skin lesions containing the bacterium, these are the only known reports in which the principal symptom was confined to the skin. Only an alertness to this type of listeric infection can reveal its true prevalence.

Conjunctivitis. Although conjunctivitis was one of the earliest recorded manifestations of listeric infection in man as a result of Anton's (5) accidental splashing of culture into a technician's eye, only a few other confirmed cases are to be found in the medical literature. Felsenfeld (60) reported listeric conjunctivitis in two poultry plant workers who were processing apparently normal chickens which harbored *L. monocytogenes* in the liver and spleen, and Beute et al. (18) isolated the bacterium from the purulent discharge of the eye of a woman with confirmed listeric meningitis. Trüb and Sauer (267) made a similar isolation from an infected newborn infant. Smeenk and Kampelmacher (244A) isolated *L. monocytogenes* type 4b from a newborn baby with purulent conjunctivitis and the same serotype from the vagina of the clinically healthy mother.

During 1949 and 1950 several Russian authors, among them Pletneva and Stiksova (204), Shemeleva (238), Shamesova, and Bilibin (24), reported some 50 cases in children and adults, many of whom had contacted rats and mice thought to have harbored the bacterium. Unfortunately, all diagnoses were based solely on serological evidence and, in many instances, the titer was not particularly high (1:100 to 1:200); there was no mention of actual isolation of the bacterium. These cases must be held with reservation for several reasons: unreliability of serological tests with *L. monocytogenes*; the established fact that rabbits with artificially produced, severe conjunctivitis do not consistently produce detectable antibodies, even though it is now known that some bacteria do invade the blood stream; and the conspicuous absence of similar reports during the intervening 12 years—a period

during which the techniques for isolating *L. monocytogenes* have been vastly improved, and which have witnessed an almost startling increase in interest in listeric infection throughout the world.

Listeric conjunctivitis in man, as in animals, is mentioned often but seldom actually observed. When conjunctivitis is seen, it is usually associated with some other form of listeric infection—meningitis or encephalitis—and is usually not the same type of purulent conjunctivitis as that which results from conjunctival instillation of culture, or as that sometimes seen in newborn infants with listeric septicemia who may have had their eyes exposed to contaminated amniotic fluid. Usually, there is only slight to marked congestion of the conjunctiva and injection of some blood vessels without conspicuous involvement of the cornea and relatively little exudate. Also, the bacterium cannot be isolated from the affected eye. It is not known what relationship the conjunctivitis has to the principal disorder, the portal of entry, the direct result of bacterial invasion, or to secondary effect of assault to the central nervous system. The only established fact is that listeric conjunctivitis can occur; yet, available information indicates that it is one of the most rare manifestations of listeric infection.

Urethritis. Having begun this section with the head and with meningitis, it seems appropriate to conclude it at the caudate end. The 1955 publication of Wenkebach (282) remains the only one recording isolation of *L. monocytogenes* from the genital organs of the human male. He isolated the bacterium from the urethral exudate of five men with gonorrhea. It is doubtful that *L. monocytogenes* played any pathogenic role in these cases. No mention was made of lesions or exudates that would arouse suspicion that these men had mixed infection. Also, if true as rumored, that all had shared a common sex partner, the most significant contribution made by the observation is the demonstration that *L. monocytogenes* may be transmitted by promiscuous sex relations. Since so little is known regarding the occurrence of *L. monocytogenes* in the male genital tract, any speculation relating to it must be based on experimental findings. According to Gray (84), the penis of rabbits permitted to copulate with does shortly after intravaginal instillation of *L. monocytogenes* was highly contaminated immediately afterward. In most instances, the bacterium could not be isolated from the penis 24 hr later and never persisted more than 6 days. There was no evidence that the male became actively infected, either locally or generally as a result of the exposure. The patients of Wenkebach (282), no doubt, represent an analogous experience under natural rather than laboratory conditions.

It has been speculated that semen may sometimes contain *L. monocytogenes*. It is not beyond the realm of possibility. Recently, Gray (89) typed two cultures: one isolated from the husband's semen, the other from the wife's vagina. Both were serotype 1. Without attempting to guess who had infected whom, it establishes that *L. monocytogenes* may be transmitted by sexual contact without apparent infection of either partner. However, since the method of collecting the semen was not given, it does not necessarily prove that the bacterium was actually in the semen. It may have been contaminated mechanically, especially if the husband had been "infected" in the manner that Wenkebach's (282) patients appeared to be.

Khalimbekov (127) claimed to have isolated *L. monocytogenes* from semen of both naturally and artificially infected sheep and goats in Azerbaijan. Also it has been isolated from the testicle of a naturally infected deer in Sweden (168) and Osebold and Inouye (186) isolated it from the testicles of an artificially infected rabbit and ram. Gray (89), and no doubt others, also isolated it from the same source from artificially infected rabbits. These findings strongly suggest not only that *L. monocytogenes* may be transmitted through semen, but also that the male, as well as the female, may shed the bacterium through the reproductive system. In view of what has been learned during recent years regarding the importance of inapparent listeric infection in women, especially during pregnancy, it may be well to attempt to determine whether the males may experience similar inapparent infections and, in this way, play an unsuspected part in transmission of the disease during intercourse.

Although *L. monocytogenes* has been frequently isolated from urine of women who gave birth to infected infants, and patients with other forms of listeric infection (136, 235, 271), there is little evidence that *L. monocytogenes* incites primary infection of any part of the urinary system. Some infected pregnant women complain of symptoms suggestive of pyelitis or cystitis, but it is questionable whether these signs indicate localized listeric infection. The bacterium's presence in the urine of such patients is, in most instances, a result of contamination of the bacterium in the genital tract, rather than reflection of true urinary-tract infection.

Other considerations. *L. monocytogenes* has been isolated from blood, throat, pharynx, and ears of apparently healthy individuals or from individuals after surgical procedures. The signifi-

TABLE 3. *Mammals known to harbor Listeria monocytogenes*

Domesticated ruminants	Zoo animals
Sheep[a]	Chinchilla (288)
Goat	Marmoset (288)
Cow	Paca (288)
Water buffalo (153, 205)	Serval (142)
	Leopard (106)
Domesticated monogastric animals	Coyote (Karstad 89)
Pig	
Horse	Feral ruminants
Rabbit	Deer (168, 262)
	Moose (6)
House pets	
Dog (85, 131, 285)	Feral monogastric animals
Cat (101, 132, 168)	Rabbit
Squirrel (35)	Gerbille (201)
Laboratory animals	Rhombomy[c]
Rabbit (164)	Merion[c]
Guinea pig (164)	Vole (11, 134, 183)
Rat (200)	Mouse (74)
Vole	Common rat
Merion (Jessen 89)[b]	Water rat[d]
Lemming (170)	Muskrat (Barns 89)
Ferret	Raccoon (69)
	Sable[e]
Fur bearing animals	Skunk (28, 188)
Chinchilla	Water shrew[d]
Fox (75, 168)	Common shrew[d]
Mink (168, 255)	Fox (170)

[a] Absence of citation indicates ample supporting references in 80, 223, 235, 249.

[b] Balozet, L. 1956. Arch. Inst. Pasteur Algerie **34**:349–354.

[c] Martinevskii, I. L. 1961. Zh. Mikrobiol. Epidemiol. Immunobiol. **32**(5):85–91.

[d] Tregubova, N. G. 1949. Veterinarya **26**(1):27–32.

[e] Eremeev, M. N., and N. D. Stepanenko. 1961. Krolikovodstvo i Zevrovodstvo, No. 4, p. 23–24.

cance of such findings is not known, but they support the concept that many healthy carriers exist in the human population. It hints of epidemiological implications which may warrant further discussion.

Although *L. monocytogenes* appears to be a somewhat insidious opportunist, it has not taken undue advantage of busy laboratory technicians who may sometimes lapse into moments of carelessness. Conjunctivitis has been the only confirmed laboratory infection (5). However, low-grade infection may be fairly common. Seeliger (89) observed rises in antibody titer following mild flu-like episodes in laboratory personnel working with *L. monocytogenes*. The gravid uterus has been found to be highly susceptible to listeric infection, and pregnant laboratory workers would avoid suspect material.

Since *L. monocytogenes* has been associated with such a diversity of disorders, some investigators would make it a "whipping boy" for all of man's unsolved ills. Parr (192) wondered whether it had a part in chronic hepatitis or cirrhosis, and it has even been suggested that it may contribute to multiple sclerosis (105). Perhaps it does, but simply because *L. monocytogenes* is so poorly understood, and sometimes behaves in such an unexpected and even unruly manner, hardly seems reason to blame it for all the unexplained diseases of man and beast. If properly treated by the stern discipline of conscientious research, it may become properly and accurately identified as a pathogen. That day may not be far away.

LISTERIC INFECTION IN MAMMALS

As shown in Table 3, *L. monocytogenes* can attack or be harbored by at least 37 mammalian species, including both domesticated and feral ruminants and monogastric animals.

As mentioned in the introduction, listeric infection has been associated with somewhat characteristic syndromes for the various animal groups. However, it is obvious that many cases of "atypical" listeric syndromes may have passed unrecognized. Recent years have exposed *L. monocytogenes* to be capricious and consistently inconsistent.

Encephalitis in Ruminants

Encephalitis of ruminants has been the most frequently recognized form of listeric infection among nonhuman animals and is of great economic importance. It affects sheep, goats, cattle, and water buffalo. In the Northern Hemisphere, it has generally occurred from late November to early May and has been most prevalent during February and March. Animals of both sexes and all ages may be affected, but it has been most common during the first 3 years of life. Gray et al. (80) reported encephalitis in 1-month-old lambs and also in an 11-year-old cow. In the USA, where there is a sharp distinction between beef and dairy cattle, beef cattle are more often affected than dairy animals. A possible explanation may be that these animals are generally exposed to more vigorous winter environment, since they are usually left outside during the entire year.

Climate appears to play a rather important role in listeric encephalitis. Gray (223) observed

an increase in the number of outbreaks 2 to 4 days after sudden drops in temperature or heavy snow falls. Gill (70) reported that, in New Zealand, the disease was most prevalent during the dry season and disappeared after rains. Khalimbekov (127), in Azerbaijan, found the highest incidence during the hot summer months. Usually outbreaks stopped when green grass was available. It may be significant that the disease has been observed most frequently in the temperate zone and seldom in the tropics. In Europe, where there appears to be a relatively high incidence of listeric infection in man, the incidence in ruminants, judged by the number of published reports, is relatively low. Whether this situation may be related to the somewhat mild European winter is an interesting, unanswered question.

It is difficult to determine the exact incidence of a sporadic, still somewhat unknown disease such as listeric infection. In Michigan the number of confirmed outbreaks in a single winter ranged from 10 to 50, depending on publicity given through veterinary medical and farm journals and radio programs. Records compiled between 1954 and 1958 by the Communicable Disease Center, U.S. Public Health Service, revealed 2,106 cases, mostly in cattle (283). Apparently, the majority of these diagnoses was made on the unreliable basis of clinical signs alone, and the figures must be taken with some reservation. With present inadequate methods for reporting animal diseases throughout the world, it is impossible to establish the true prevalence of listeric infection.

The disease in sheep and goats has been extremely acute, and death may occur within 4 to 48 hr after first signs. A few animals have survived for several days. Recovery has been rare among sheep and goats that had definite signs of infection. Mortality ranged from 3 to 30% or more. Kornilova (132) in Novosibirsk recorded losses as high as 39 of 40 in one flock and 59 of 60 in another.

In cattle, the disease has been more chronic, and most cows survived from 4 to 14 days after first signs appear. Spontaneous recovery has been frequently observed.

Acute outbreaks in which deaths were sudden and a high percentage of the herd was involved are rare. Usually no more than 8 to 10% of a herd were affected. Although some cows responded to sulfa or antibiotic therapy, those animals which recovered spontaneously often had long-lasting brain damage such as permanent torticollis, incoordination, or various degrees of paralysis. Such animals are unsightly and unable to compete with the rest of the herd. Gray and Moore (81) found alterations in the brains of six beef cattle slaughtered 1 year after apparent recovery, but failed to isolate *L. monocytogenes*. Since some lactating animals with listeric encephalitis may excrete the bacterium through the milk for long periods, recovered dairy cattle present not only aesthetic disadvantages but also genuine public health hazards.

Attention has been focused recently on a more chronic nonfatal form of listeric encephalitis of sheep. It usually occurred simultaneously with the less acute form. Many of the animals were reported to recover, but, until reliable methods for confirming antemortem diagnoses are devised, it cannot be determined whether such sheep actually have a listeric infection. Gray and co-workers (89) cultured spinal fluid from some of these animals on tryptose agar, but failed to isolate *L. monocytogenes*. The method also failed with animals that subsequently died from confirmed listeric encephalitis. However, Eveleth et al. (58) succeeded in isolating *L. monocytogenes* by inoculating embryonating chicken eggs with spinal fluid from a living sheep that had signs of encephalitis. It may be that wider application of the egg technique would result in more satisfactory antemortem diagnostic detection. It is striking that, in contrast to ruminants, *L. monocytogenes* can be isolated with comparative ease from spinal fluid of man with meningitis. It may be another reflection of basic difference in disease process in these two species. Since in ruminants there is a localized encephalitis with the lesions confined primarily to the medulla, there may be less opportunity for the bacterium to enter the spinal canal than in the more disseminated meningitis in man. Animals with nonfatal listeric infection, or which recover after therapy, may be carriers for long periods, and should be eliminated as quickly as possible.

Symptoms in all ruminants are similar, and differ only in severity. At onset, the infected animal usually separates itself from the rest of the herd. It appears depressed, confused, and indifferent to surroundings. Incoordination and torticollis follow. Often, intermittent twitching and paralysis of the facial and throat muscles and the tongue, which usually protrudes, interfere with swallowing, resulting in marked salivation. One or both ears may be drooped. Frank convulsions are rare. Often there is strabismus and conjunctivitis, and the animal may appear blind. Marked nasal discharge, anorexia and temperature of 108 F or more are common. In the early stages, the animal tends to crowd into corners or lean against stationary objects as if unable to stand unsupported. If the animal walks, it often moves in a circle, always in the same direction.

However, not all infected animals circle, and the often used term "circling disease" is misleading and should be avoided. In the terminal stages, the animal falls and cannot get up without assistance. When it is down, there are generally rapid, deep abdominal breathing, involuntary, aimless running motions, and constant fine tremors of the head. At this stage, the animal attempts to eat, or at least makes chewing motions, until the moment of death. This symptom is so characteristic that it is almost pathognomonic. Viciousness is not seen except occasionally in cattle. It is unlikely that all of these signs would appear in a single animal. Many cattle are unable to swallow. They may survive relatively long but seldom recover.

Antemortem diagnosis of listeric encephalitis is virtually impossible because of lack of a satisfactory diagnostic test(s). The hemogram usually has no marked variation from normal. Sheep may have polymorphonuclear leukocytosis. The peripheral monocytosis, often found in monogastric animals, is not characteristic of ruminants. Although reported a few times, there is serious doubt, based on artificial infection observations (179), that the monocytic response can occur in ruminants. It may indicate fundamental physiological differences in the reaction of ruminants and monogastric animals to infection with *L. monocytogenes*. The various serological tests are difficult to interpret. Many apparently normal animals have relatively high titers against *L. monocytogenes* but their significance remains undetermined. Today, listeric infection can be confirmed only by isolation and identification of *L. monocytogenes*.

Usually there are no detectable gross lesions in animals that die with listeric encephalitis. Slight clouding or pin-point grayish-white foci of the meninges may be rarely observed. There may be slight congestion of the brain, and some sheep brains have marked congestion. Usually, there is an increase in the amount of cerebralspinal fluid. Lesions of other viscera are rare, but occasionally fatty liver, duodenitis, or pulmonary edema are seen. Focal hepatic necrosis has been reported in adult sheep, but not cattle.

Microscopic lesions have been confined primarily to the pons, medulla, and anterior spinal cord. Both white and gray matter may be involved. The primary lesions, marked perivascular cuffing with varying degrees of focal necrosis, appeared to develop in the brain substance, but may extend into the meninges. The perivascular cuffs consisted mainly of mononuclear cells. In sheep and goats, the necrotic foci contained a preponderance of polymorphonuclear leukocytes and sometimes appeared purulent, with complete disintegration of the parenchyma. There was edema, hemorrhage, neuron degeneration, neuronophagia, and the blood vessels were congested, frequently containing thrombi; degenerative changes of the endothelial lining were also observed.

In cattle, the perivascular cuffs have been much smaller and the focal lesions usually were limited to edema and small collections of microglial cells and lymphocytes. Rarely were lesions as extensive as in sheep, re-emphasizing the more chronic nature of the disease among cattle.

L. monocytogenes was never found in the perivascular cuffs, but could be demonstrated quite readily in the focal lesions. The bacteria occurred most frequently near the periphery of the lesion and were either extra- or intracellular. In cattle, they tended to occur singly or in small clumps, while in sheep dense plaques may be formed.

Clinical signs alone are not satisfactory as diagnostic criteria. Deaths subsequent to similar signs in the same herd or flock after positive diagnosis may be presumed to be listeriosis. However, even this has often proved unreliable, particularly in sheep flocks where encephalitis, enterotoxemia, and ketosis may occur simultaneously and be distinguished only at necropsy. In listeric encephalitis, there are no distinctive lesions such as the fatty liver of ketosis and the characteristic hemorrhages of enterotoxemia. Acetone may be found in the urine of animals with listeric encephalitis as in ketosis.

Listeric encephalitis in cattle may mimic rabies, poisoning, acute gastroenteritis, ketosis, Aujesky's disease, or viral encephalitis.

In areas where rabies is endemic, it is difficult to distinguish between it and listeric encephalitis merely by clinical syndromes. Perhaps a decade ago the late Serge Lensen of the Michigan Department of Health, Bureau of Laboratories, casually mentioned that sometimes mice inoculated with bovine brain from rabies suspects died of bacterial septicemia. Almost jokingly Gray said, "Why don't you send those *Listeria* cultures to me next time?" So he did—and they were just that—cultures of *L. monocytogenes*. The episode was repeated several times but it remained for March (154) to first put such observations into print. It led him to suggest that intracerebral inoculation of mice might be a better method than use of nonliving media for attempting to isolate *L. monocytogenes* from the bovine brain. It also alerted other laboratories not to discard mice which died 48 to 72 hr after inoculation without culturing them. In this

chance way, many cases of listeric encephalitis have been salvaged.

L. monocytogenes has been isolated from a rabies-positive bovine brain by Gray et al. (80), a feral fox brain by Avery and Byrne (10), and from brains of foxes suspected of having rabies by Scholtens and Brim (229*a*).

As early as 1941, Stenius (256) in Finland and later Beller and Zeller (17) in Germany suggested, because of similarity of some signs and lesions, that there might be a relationship between *L. monocytogenes* and malignant catarrhal fever of cattle. With the exception of some Russian investigators (249), particularly Stolnikov (257), these reports received scant attention. Stolnikov (257) eventually concluded, as had Stenius (256), that these diseases are distinctly different, although they can occur simultaneously in the same herd, and sometimes even in the same animal.

Although it is difficult to isolate the bacterium from the living animal, it is relatively easy to isolate it from the medulla oblongata of animals that die. *L. monocytogenes* has a marked tendency to localize in this portion of the brain. The probability of successful isolation is greatly enhanced if cultures are prepared from this area. With employment of the proper method, primary cultures from sheep and goat brains usually yield the bacterium. However, many cow brain suspensions require several weeks or even months of refrigeration before a positive culture can be obtained. Using the maceration and refrigeration technique (78, 82), Gray et al. (85) isolated *L. monocytogenes* from more than 140 specimens where the disease was suspected and where tissue sections revealed perivascular cuffing and focal necrosis characteristic of listeric encephalitis. The number of colonies that developed from bovine brains was always small compared with that of ovine or caprine brains. This difference in colony numbers may further reflect the more chronic nature of listeric encephalitis in cattle than in sheep and goats.

It is a striking but almost completely ignored fact that listeric encephalitis has never been observed in lambs or calves before the rumen has become functional. In these animals, infection has been always manifested by septicemia similar to that seen in other monogastric animals. Whatever the explanation for this may be, it reflects a basic physiological difference that dictates that ruminants are more susceptible to encephalitis, whereas monogastric animals, including ruminants before the rumen is functional, are more susceptible to septicemia. The detection of this difference might be valuable not only to the growing accumulation of academic knowledge, but also to afford a better understanding of still obscure pathogenesis of all forms of listeric infection.

Another puzzler for those fascinated by pathogenicity problems is the often-made observation that listeric infection is more common among ruminants fed silage than among those fed other rations. Krüger (138*a*) reviewed the literature on these observations. He also reported his studies on the occurrence of *L. monocytogenes* in different silages and its etiological significance in listeric infection in sheep in Eastern Germany. This line of study has been continued by Lehnert (147*a*) in Eastern Germany and by Dijkstra (51*a*) in the Netherlands. Gray (88) isolated *L. monocytogenes* from mice injected with aqueous extracts of oat silage and subsequently isolated the organism directly on tryptose agar from the aqueous extract. Palsson (191*a*) reported that Gislason and Vigfusson in Iceland had isolated *L. monocytogenes* from guinea pigs injected with grass silage extracts and also directly from the extracts in 1945—but unfortunately their findings were not published. "Votheysveiki" or silage disease has been a serious problem in sheep in Iceland since the beginning of the century. Grass silage as prepared in Iceland is often of poor quality.

Krüger (138*a*) postulated that *L. monocytogenes* is widely distributed in the soil and on the plants in certain districts in Eastern Germany. During the fermentation in silage making, the *p*H reaches 3.8 to 4.2, which is bacteriostatic for *L. monocytogenes*. The optimal conditions for fermentation do not exist along the sides of the silo nor on the top. In these areas of the silo, the *p*H of the silage is much higher. *L. monocytogenes* was isolated from poor quality silages from these areas of the silo where *p*H values of the silage were from 5.7 to 8.9. Often this poor quality silage also contained abnormally high numbers of *Aerobacter*, *Pseudomonas*, and *E. coli*, as well as molds. Krüger believes that the feeding of spoiled silage is a frequent cause of listeric infection in sheep. Lehnert (147*a*) postulated that feeding of silage containing *L. monocytogenes* to cattle and sheep produces a latent or inapparent infection in a large number of these animals, often approaching 100% of the animals in a herd. However, clinical signs seldom appear in cattle, and only a small number of sheep develop listeric encephalitis. Dijkstra (51*a*) in the Netherlands isolated *L. monocytogenes* from 30% of samples of silage from 140 farms on which a case of listeric abortion in cattle had been reported.

Listeric Septicemia in Ruminants

Primary listeric septicemia in adult ruminants has been relatively rare, at least in the USA. It is most frequently reported from southeastern Europe and the Soviet Union. Sheep are the most usual victims, but it also has been observed in feral deer in Germany (262) and Sweden (168). The animals had general weakness, inappetence, and respiratory distress. Mortality was not as high as that for listeric encephalitis. At necropsy, there was focal necrosis, usually involving the liver and spleen. The bacterium could be isolated most easily from these organs, or blood. The disease has seldom been reported in the USA, and, when seen, was usually in conjunction with encephalitis. However, outbreaks involving several hundred animals in a single flock have been observed in Idaho (89). Although morbidity was high, mortality was low. Both ewes and lambs were affected. An interesting feature of at least one outbreak was the development of encephalitis among some recovered animals that were put on mountain pastures. Perhaps the situation is more common than generally suspected but has escaped the attention of investigators.

Listeric Septicemia in Monogastric Animals

The above title is somewhat misleading and it may have been better to omit it. However, the septicemia form has been the most usual manifestation of listeric infection in this group of animals, although exceptions are more common than among ruminants. Many monogastric animals have had meningoencephalitis, with or without septicemia.

In general, signs and lesions have been similar among the various animal groups. Death could be sudden with no indication of previous illness, or the animal may have been generally depressed and weak with a tendency to lie quietly. This condition may persist for several days and be accompanied by dyspnea, slobbering, nasal discharge, and lacrimation. Short periods of convulsions have occurred, and many animals attempted to eat until death. Limited blood studies indicated monocytosis to be common.

The most consistently seen lesion at necropsy was focal hepatic necrosis. It ranged from few pinpoint foci to almost complete studding of the organ. Occasionally, similar foci were seen in other viscera. The mesenteric lymph nodes may be congested, soft, and swollen, the adrenals may be enlarged, blood-tinged fluid containing fibrin appears in peritoneal and pleural cavities, and enteritis and rarely myocardial necrosis occur. At times, gross lesions were completely absent or limited to slight swelling of the liver and spleen and a few superficial hemorrhages on these organs.

Lesions are histologically characterized by focal necrosis with infiltration by mononuclear cells and some polymorphonuclear leukocytes. Sometimes infiltration with the latter was so great as to give the appearance of purulent reaction. Usually, the necrotic areas were well demarcated with little involvement of the adjacent tissue. *L. monocytogenes* may be seen either within or outside the cells and is most numerous at the periphery of the lesion. In severe cases, the entire organ may be involved with no normal tissue remaining.

Signs among monogastric animals with listeric meningoencephalitis may be indistinguishable from those of any other meningoencephalitis. At necropsy, gross lesions may be completely absent or there may be slight clouding of the meninges with or without the presence of small foci of necrosis. This group is so large (Table 3) that it seems best to subdivide it into reasonably smaller groups. Since we have been discussing domesticated food-producing animals, we may as well continue with them—even if it leads over the sty into the pig pen.

Swine. With the exception of the Soviet Union, where listeric infection appeared to be quite common in pigs in some parts of Siberia (132, 249), the disease is relatively rare. However, *L. monocytogenes* has been known to produce septicemia, meningoencephalitis, localized internal abscesses, and poxlike skin lesions in swine. In the USA, many pigs had signs suggestive of listeric encephalitis, but when necropsied the bacterium could not be isolated. Neither did the histological lesions resemble those typical for listeric infection. The condition appears to be a form of hog cholera erroneously diagnosed clinically as listeric infection. There are several reports of listeric infection and hog cholera occurring simultaneously in the same animal. *L. monocytogenes* has also been isolated from pigs with swine erysipelas or swine influenza. These mixed infections appear to be most common in southeastern Europe.

The most common form of listeric infection in swine is septicemia, during the first few weeks of life, characterized by focal hepatic necrosis. *L. monocytogenes* can be isolated from the liver. Although the disease may be relatively rare in pigs, the somewhat frequent isolation of the bacterium from apparently normal pigs or from those which obviously died from some other cause, strongly suggests that they may play an important

part in transmission of the disease or that swine may be important reservoirs of the bacterium.

The widespread use of pig feeds containing antibiotics may be a factor in the low prevalence of the disease in the USA.

House pets. Listeric infection does not seem to be a serious problem among house pets, but has been observed in dogs, cats, and a pet squirrel (35). Primary listeric infection of dogs has been reported at least six times. During a period of several months, Cox (41) reported isolation of *L. monocytogenes* from the medulla of four dogs which showed signs suggestive of rabies. However, all tests for rabies were negative. Chapman (34) observed a dog with signs of meningitis which showed a monocytosis of 25% three days before death. Necropsy confirmed the diagnosis, and the bacterium was isolated from the brain. Garlick et al. (68) reported seven cases of listeric meningoencephalitis among 18 Weimaraners in a single kennel. The first dog became ill 23 days after surgical removal of cartilaginous growth from the base of the skull. The second dog became ill 5 days later, and the remainder began developing signs within the following 10 days. What part, if any, the surgical procedure had in inciting the outbreak, unfortunately, must remain unknown. Also unknown is the complete history of an intriguing outbreak of a distemper-like disease among sled dogs in Labrador. Vaccination against distemper had little effect on the course of the disease in most areas. *L. monocytogenes* was isolated from some of the brains of several dogs sent to the Animal Diseases Research Institute, Hull, Quebec. Practically no rabbits were seen on the Labrador coast during that year, 1955, and those that were caught were thin with patchy loss of hair. Sled dogs in the area feed on rabbits and lemmings, both of which are known to harbor *L. monocytogenes*. At the same time, some Eskimos had vague infectious mononucleosis-like symptoms and gram-positive rods resembling *L. monocytogenes* were seen in the spinal fluid of one. However, the true significance of all this remains as silent and unknown as the land in which it occurred.

Eveland and Griffes (91*b*) reported illness in two dogs associated with a man with listeric infection. One dog died from convulsions of an undiagnosed cause. The second dog, that regularly slept with the patient, became blind. When this dog was autopsied, organisms with typical fluorescence were demonstrated in liver impression smears and sections stained with *L. monocytogenes* type I fluorescent-antibody conjugate.

Although the number of reports of listeric infection of dogs is small, there is reason to suspect that the incidence might prove to be much higher if the brains of all dogs showing nervous signs were submitted for culture of this bacterium.

The common domestic cat, despite its love for feasting on birds and small ground game which may harbor *L. monocytogenes*, and its reputation for nocturnal promiscuous adventures, should be sufficiently cunning to slink away from contact with the bacterium. Nevertheless, some have been outwitted (101, 132, 168), As indicated in Table 3, the domestic cat's larger relatives, carefully sheltered in zoos, have not been quite so fortunate. The latter may have important epidemiological implications, since it is known from laboratory studies that domestic cats are highly resistant to artificial infection (75). Several species of small ground game have developed listeric infection after incarceration for use as laboratory animals (170).

Domesticated rodents and carnivores. Table 3 indicates that *L. monocytogenes* may attack or be harbored by a number of small animals raised for food, laboratory use, or pelts. With few exceptions, infection has been manifested by septicemia. In addition to the symptoms already enumerated, there may be sharp cries as if in pain. Often marked diarrhea has been seen among affected chinchillas.

At necropsy, the stomach is usually filled and the most consistently seen lesion is focal hepatic necrosis. Many guinea pigs have conspicuous, diffuse myocardial necrosis, which is almost characteristic of this species.

Many domestic rodents, particularly rabbits and chinchillas, may have almost pathognomonic hemorrhagic and necrotic metritis, the latter with or without history of abortion. Usually, these animals are pregnant or only a few days postpartum. Schoop (231) reported that of 130 rabbits which died during a 14-day period, all were females, and most were pregnant or postparturient. Some infected animals reported by Murray et al. (164) were pregnant or in the postpartum period. In view of the apparent extreme susceptibility of the reproductive system of the gravid subject to listeric infection, it is quite possible that this system is actually the primary site of active infection.

Although many reports have mentioned signs suggestive of central nervous system disturbance, localized meningoencephalitis has seldom been reported. However, such involvement has been observed in rabbits, lemmings, and chinchillas. Signs have included torticollis, incoordination, loss of equilibrium, and rolling. These signs persist for a few days to several weeks. Traub (265)

found no detectable gross lesions at necropsy of affected rabbits. Histologically, the lesions were confined to the midbrain and anterior portion of the cervical spinal cord, and were characterized by focal necrosis and perivascular cuffing. Only occasionally were focal lesions seen in the meninges. Unfortunately, of the other authors who mentioned nervous signs among rodents, few examined the brain. Only Shalkop (239) reported small collections of round cells in the brain cortex of three chinchillas.

Outbreaks have generally been self-limiting among domestic or captive rodents, and only a few animals are involved in an outbreak. Exceptions have been reported by Murray et al. (164), who observed 78 deaths among an undisclosed number of laboratory rabbits and guinea pigs, and by Schoop (231), who recorded 130 deaths in 14 days in a colony of 500 rabbits due to listeric septicemia. Chinchillas appear to be most susceptible of all species. Jacotot (113) recorded 51 deaths in a group of 80; Gray (89), 35 deaths among approximately 100 chinchillas and complete destruction of a herd of 35 over an 11-week period.

Feral animals. From Table 3 and the publication of Gray (91*c*), it is apparent that *L. monocytogenes* has no particular preference for domesticated animals over feral animals of various size, species, and habits. The clinical symptomology in naturally infected feral animals is obscure, since most are either found dead or captured in traps. When observed shortly before death or capture, signs were usually weakness and depression, suggesting that signs simulate those seen in domesticated animals. The report of Vallee (272) suggests that abortion may also occur among wild rabbits. Vallee isolated *L. monocytogenes* from the liver of a weak hare which had bloody vaginal discharge. The uterus contained a mummified fetus, or there was evidence indicating that the animals had recently either aborted or given birth to young.

Although the disease appears to be self-limiting among feral animals, eating infected carcasses by carnivorous animals or birds may constitute a mode for further spread of the bacterium. It has been suggested that listeric infection may be involved in the so-called lemming crashes in northern Canada (170). Although efforts to establish a connection have failed, it has been demonstrated that apparently normal lemmings may be carriers (170). The possibility was first suggested by the development of listeric infection in lemmings after capture, transportation, and incarceration (170); confirmation came from production of listeric septicemia in an apparently normal lemming after administration of large doses of cortisone (170). Similarly, it has been suggested by both Bolin et al. (28) and Osebold et al. (188) that listeric infection may have a role in the periodic decline of skunk and racoon populations.

Many feral mammals from which *L. monocytogenes* has been isolated were apparently healthy carriers (11, 94, 134, 183). Most isolations were made incidental to surveys on prevalence of *Pasteurella tularensis* among ground game. *L. monocytogenes* actually was isolated from mice inoculated with organ suspensions of the trapped mammals. These reports make no mention of either illness or of lesions in organs which were macerated and injected into mice. Only Olsuf'ev and Emel'ianova (183) mentioned isolation of both *P. tularensis* and *L. monocytogenes* from the same organ pool. The reports suggest that *L. monocytogenes* is rather widely distributed among small ground game and that they may contribute to spread or perpetuation of the disease in certain areas.

With known susceptibility of domesticated ruminants to listeric infection, it is not surprising that *L. monocytogenes* has also been found in deer. However, it is a little surprising that it should occur in septicemic rather than encephalitic form. Thamm (262) isolated *L. monocytogenes* from a deer that died in a small damp forest heavily grazed by sheep and cows. Listeric infection was endemic among sheep of the area. It is impossible to determine whether the sheep were infected from the deer, or whether the deer merely suffered chance infection from the sheep, or whether there was no connection at all.

Nilsson and Karlsson (168) reported chronic purulent orchitis and subacute fibrinopurulent pericarditis with isolation of *L. monocytogenes* from the testicle, liver and spleen in one of two deer. The other deer had acute purulent bronchopneumonia and acute septic splenitis; the bacterium was isolated from the liver and spleen.

LISTERIC INFECTION IN FOWL

L. monocytogenes has been isolated not only from a large number of mammalian species, but also from at least 17 different avian species as indicated in Table 4. Included are domestic fowl as well as possible house pets, zoo specimens, game birds, and a predator, emphasizing that *L. monocytogenes* is widely distributed in nature.

Listeric infection in birds has been reported from all continents except Africa and Antarctica. It is most common in the temperature zone of both hemispheres in areas ranging from the Arctic to Ceylon. In the United States, it has been

TABLE 4. *Fowl known to harbor Listeria monocytogenes**

Chicken	White grouse (Stenberg, 89)
Goose	Partridge
Duck	Snowy owl
Turkey	Crane (Stenberg, 89)
Pigeon	Whitethroat (116)
Canary	Lorikeet (288)
Parrot	Dove (288)
Eagle	Pheasant†
Wood grouse	

* Modified from Gray (86) except where indicated by other reference.

† Lucas, A. 1961. Bull. Offic. Intern. Epizoot. 55:879–887.

reported from New York to California, and the true incidence is certainly much higher than the nine published reports indicate. Published reports suggest that the Netherlands has the highest incidence, but published reports are not a reliable measure of incidence in Sweden, for example, where *L. monocytogenes* has been isolated from chickens more than 110 times in a 9-year period, yet only one report appears in the literature (168). In Norway where listeric infection in mammals is fairly common, *L. monocytogenes* has been reported only once from a bird: a canary (86).

As in mammals, young fowl appear to be most susceptible. Outbreaks are sporadic, and mortality may vary from a few to as much as 40% of the flock. There are no pathognomonic signs or lesions. Paterson (86) reported sudden death of adult chickens while young birds had a slow wasting before death. In contrast, Csontos et al. (42) stated that 2- to 3-week-old geese died suddenly, often within a few hours after onset of signs. Geese 6 to 8 weeks old survived somewhat longer and had torticollis, spasms, and other nervous signs. Some naturally infected birds have had monocytosis, but the finding is not consistent and has little diagnostic value. Since this change is common among artificially infected birds, it may occur in birds with primary listeric infection, but not in those in which *L. monocytogenes* plays only a secondary role.

Although *L. monocytogenes* has been often isolated from fowls with other disorders, there are numerous reports of primary listeric infection. The disease has been most commonly manifested by septicemia, and the bacterium can be isolated from most of the viscera, particularly liver or spleen and occasionally brain. The most conspicuous lesions are massive areas of myocardial degeneration with marked engorgement of the cardiac vessels, pericarditis, and increased amount of pericardial fluid. In some instances, the heart may show varying numbers of well-defined grayish-white foci of necrosis ranging from pinpoint to several millimeters in diameter. The cardiac lesions are often erroneously considered not only specific but also characteristic for listeric infection in fowl. However, not all birds develop this, and similar lesions are found in other species, particularly artificially infected guinea pigs. Focal hepatic necrosis without cardiac alteration has also been frequently seen. Sometimes similar foci occur in the spleen and lungs. Other lesions encountered include splenomeglia, nephritis, peritonitis, enteritis, ulcers in the ileum and ceca, necrosis of the oviduct, generalized or pulmonary edema, inflammation of the air sacs, and conjunctivitis. In acute cases, the necrotic lesions may tend to be less marked, and only congestion and few small hemorrhages throughout the viscera are seen.

Infrequently predominating signs and lesions have been confined to the central nervous system, e.g., in chickens (86), a goose (12), and a turkey (86). A most conspicuous sign was marked torticollis. In all instances, *L. monocytogenes* was isolated from the brain. At necropsy, only geese showed myocardial necrosis. Bandaranayake (12) mentioned histological changes of the brain. He observed marked congestion, but no foci of infiltration. These reports establish that listeric infection in fowl may be manifested not only by septicemia, but also meningoencephalitis, as in mammals.

There is a paucity of information of histopathological changes in the tissues of naturally infected fowl. This situation may be due partially to the fact that *L. monocytogenes* has often been isolated rather incidentally from birds obviously suffering from other disorders. The few existing reports are fragmentary and conflicting, and it is impossible to reconstruct an accurate picture. If laboratory findings can be accepted as suitable substitutes, Pallaske (86) has given the best account. His intravenously exposed chickens developed myocardial degeneration. Focal or diffuse necrosis was the most consistent alteration, providing the birds lived for 5 to 7 days. Sometimes, the lesions were characterized by edema, marked proliferation of histiocytes, and infiltration of monocytes, lymphocytes, and plasma cells. These often formed a marked "cuff" around the capillaries and small vessels. In Gram-stained sections, the bacterium was always found at the periphery of the lesion. There was also early focal necrosis in the liver and spleen, but lesions were not detected in the kidneys or brain. However, Traub (86) observed

low-grade purulent meningitis in a hen which died 15 days after intravenous exposure and which showed signs of nervous disturbance.

It has often been suggested that *L. monocytogenes* plays only a secondary role in disease of fowl. In support, it has been associated with salmonellosis, Newcastle disease, fowl pest, coryza, coccidiosis, worm infestations, mites, enteritis, lymphomatosis, ovarian tumor, and lowered resistance due to inbreeding or hatching of imported eggs. The bacterium also has been isolated from the spleen of apparently normal chickens (86, 168) and the intestinal tract of an apparently normal snowy owl shot in the Arctic region of Quebec (86). This owl may have fed on lemmings, which are known to harbor *L. monocytogenes* (170). The blue eagle from which Schulze (86) isolated *L. monocytogenes* did not appear ill immediately before death. However, it had been treated over a long period for an abscess of the abdominal wall. It is known that *L. monocytogenes* may be associated with abscessation, which may have been the primary focus of infection.

The fact that listeric infection among fowl has often been complicated by some other disorder, coupled with the relative difficulty of artificially infecting healthy birds, suggests that they possess a rather high degree of natural resistance to the disease. Segre et al. (86) failed to incite infection in 1- to 2-week-old chicks when antimetabolites were administered in an attempt to alter the tricarboxylic acid cycle. The canary appears to be the most susceptible, and has been employed successfully as a laboratory animal.

As in most other forms of listeric infection, the mode of spread among birds is not known. Present evidence suggests that most birds become infected by pecking contaminated soil, fecal material, or dead mammals.

Although the presence of necrotic lesions in the oviducts of some hens with listeric infection suggests the possibility that eggs may contain *L. monocytogenes*, this has never been confirmed.

The present widespread use of poultry feeds containing antibiotics may have prophylactic value against listeric infection in domesticated birds. Csontos et al. (42) gave feed containing "Aurofac" at the rate of 5 to 20 mg of chlortetracycline per kg of body weight to 360 birds during a large outbreak of the disease among young geese. Only 1.1% of these birds died, while 24% of 100 control birds died during the same 2-week observation period. Ever since feeding of antibiotic feeds was initiated several years ago, reports of the disease in poultry have become rare. Hence, it seems there is no further need for a vaccine for poultry as once suggested by Annagiv (4).

LISTERIC INFECTION OF FISH

Stamatin et al. (252), in Romania, isolated *L. monocytogenes* from the viscera of pond-reared rainbow trout. They had been fed meat from a donkey which died of an undetermined cause. The fish showed listlessness interrupted by brief periods of agitation, inappetence, apparent blindness, blackened integument, and bloody discharge from the anus, particularly by the females. Mortality was about 50%. Gross lesions included generalized congestion throughout the viscera, serous fluid in the pericardial sac, and gas and viscid liquid in the terminal portion of the intestinal tract. Histologically, there were vacuolation and accumulations of hyalin material in the hepatic cells, infiltration of bile ducts with lymphocytes and monocytes, distortion, desquamation and granular degeneration of the epithelium of the kidney tubules, hemorrhage and massive infiltration of lymphocytes and monocytes, but few polymorphonuclear cells in the intratubular spaces. The disease could be transmitted to trout but not to carp by intramuscular or intracranial inoculation.

The cultures isolated from these fish have been studied by Seeliger, Murray, and Gray. Although they proved somewhat different from typical cultures of *L. monocytogenes*, there appears to be justification for considering them as valid members of the species. For the present, the true significance of this report can only be speculated. The fish appear to have been infected by the donkey meat, but unfortunately this can never be established.

Fish are not the only aquatic forms of life to be infected by, or at least to harbor *L. monocytogenes*. Shlygina (241) studied cultures of the bacterium isolated from crustaceans gathered in the same stream from which Olsuf'ev et al. (184) isolated the bacterium from the water. It is doubtful that the crustaceans were actually infected. However, this report establishes that *L. monocytogenes* may be carried and spread by aquatic life.

EPIDEMIOLOGY AND PATHOGENESIS

The epidemiology of listeric infections is poorly understood. Gray (70) reviewed the literature on this subject, and in the summary of his paper stated: "Listeric infections in both animals and man are more prevalent than published reports indicate. Listeric infection is not necessarily an acute highly fatal disease but may be manifested by low-grade, even inapparent infections which pass undetected in nonpregnant subjects but lead to infection of the uterine contents of the pregnant subject. *Listeria monocytogenes* may

occur in silage in sufficient numbers to produce infection in ruminants. Ticks and other vectors may contribute to dissemination of the bacterium, but their importance remains undetermined. Healthy carriers exist among human and animal populations and these appear to play a predominant role in perpetuation and transmission of the disease. There is no evidence to substantiate the claim that listeric infection in man results primarily from direct contact with infected or carrier animals. Many human subjects may be carriers and when physical and/or physiological stress undermines host resistance, active infection may result. Human neonates appear to be infected *in utero* as a result of inapparent infection in the mother."

Further information on the epidemiology and pathogenesis of listeric infections, the use of vaccines and the incidence of listeric infections in man and animals in several countries is available in the papers and discussions presented at two symposia. The first was held in Giessen on 27 and 28 June 1957 (223), the second in Bozeman, Montana, on 29 to 31 August 1962 (261). In addition, Seeliger (235) has published a monograph on listeriosis. A bibliography of approximately 1,550 references has been deposited as Document number 7549 with the ADI Auxiliary Publications Project, Photoduplication Service, Library of Congress, Washington, D.C.

With recent improvements in the techniques for isolation of *L. monocytogenes* from feces, soil, silage and sewage sludge, information has accumulated on the reservoirs of the organism in nature together with suggestions shedding further light on the epidemiology of listeric infections in man and animals. Sandvik and Skogsholm (227*a*) isolated *L. monocytogenes* from mice which had been injected subcutaneously with a suspension of sheep feces. Dkjkstra (51*a*) used mice in his isolation scheme for *L. monocytogenes* from silage and feces but injected the mice twice within 1 week between the first and second injection. When the mice died, or were sacrificed, 4 to 5 weeks after the first injection, suspensions were made of the liver and spleen and stored at 4 C. From these, transfer inoculations to selective bacterial media were made at regular intervals. Lehnert (147*a*) found selective media containing potassium thiocyanate useful in isolating *L. monocytogenes* from feces, silage, and the nasal mucosa of sheep. Bojsen-Møller (27*a*) combined prolonged storage at 4 C with the addition of polymyxin to the medium to isolate the bacterium from human feces. Seeliger et al. (237*a*) used the simple procedure of diluting human fecal specimens 10^{-4} with physiological saline and streaking this highly diluted suspension on the surface of sheep blood-agar to increase greatly the cultural isolations of *L. monocytogenes* from feces, garden soil, and activated sewage sludge.

Bojsen-Møller (27*a*) found *L. monocytogenes* in the feces of 3 of 7 (42.9%) human patients under 1 month of age with listeriosis, in 4 of 18 (22.2%) patients over 1 month of age with listeriosis, in 10 of 45 (22.2%) household contacts of listeric patients, in 6 of 620 (1.0%) gastroenteritis patients, in none of 200 patients in a pediatric ward, in 12 of 1,040 (1.2%) patients in a medical and gynecological ward, but in 55 of 1,150 (4.8%) of symptom-free slaughterhouse workers and employees. Bojsen-Møller postulates that animals may be a primary reservoir of *L. monocytogenes*. Larsen (144*a*), using Gray's cold incubation and oblique-lighting technique, isolated *L. monocytogenes* 35 times from animals and 4 times from sewage from a total of 2,940 samples tested. Animals infected were cattle, sheep, mink, chinchilla, dog, mouse, chicken, starlings, and house sparrow.

Lehnert (147*a*) found that listeric encephalitis in sheep is not an isolated infection of the brain but a generalized infection in which *L. monocytogenes* may be present in the liver, spleen, kidney, and mesenteric lymph node, nasal mucosa, and feces. Seeliger et al. (237*a*) isolated *L. monocytogenes* from the feces of a woman who had aborted four times, from the feces of her husband, from the garden soil, and from activated sewage sludge used as fertilizer on the garden. Seeliger *et al.* suggested a possible listeric infection cycle in man: infected human feces → soil contamination → soil or fecal contamination of fresh vegetables → oral infection in human beings. In ruminants, a possible cycle might be: infected animal → shedding of *L. monocytogenes* with the urine and feces → organism persisting in soil, manure, dust, or filth → propagation under favorable environmental conditions such as neutral or alkaline, but not well-fermented, silage → oral infection through silage.

Among the recent studies on the pathogenesis of listeric infection, the work of Sword (259*b*) and Wilder and Sword (282*a*) suggests that the in vivo growth of *L. monocytogenes* may be enhanced by the presence of elevated iron values. Mice treated with 80 μg of Fe^{+++} or Fe^{++} per day for 3 days had a mean reduction in the LD_{50} of over 2×10^3 organisms. *L. monocytogenes* grew more rapidly and in greater numbers in the liver and spleens of mice treated with iron. Experimentally induced hemolytic anemia resulted in increased susceptibility of mice to the bacterium.

Stewart et al. (256*a*) found increased suscepti-

bility of mice to airborne infection with *L. monocytogenes* after continuous exposure to γ radiation delivered at 1.0 to 1.5 rad per hour.

For several years Dr. Gray's laboratory, in cooperation with the Communicable Disease Center of the U.S. Public Health Service, did serological typing of isolants of *L. monocytogenes*. To gain a better understanding of the epidemiology and pathogenesis of listeric infection, a questionnaire asking for the patients' clinical histories and epidemiological information was sent to each laboratory submitting cultures for serotyping. These data on 539 bacteriologically confirmed cases of listeric infection in man in the United States which occurred in the 30-year period between February 1933 and February, 1963 have been published (71). The serotyping of 417 cultures showed: type 1, 137; type 3, 3; type 4a, 4; and type 4b, 273. This important work on the serological typing is being continued by Joseph H. Schubert, Chief, Microbiology Diagnostic Unit, Laboratory Branch, Communicable Disease Center, Public Health Service, Atlanta, Ga.

CONCLUDING REMARKS

Many of the unsolved problems of *L. monocytogenes* and listeric infection were alluded to in Gray's closing summary statement to the Second Symposium on Listeric Infection (261):

1) Listeric infection is widespread in both domesticated and wild animals, but few definite figures are available on its incidence. It is not known with certainty whether affected or carrier animals constitute a source of infection for man.
2) There appears to be need for a vaccine for use in animals in some areas. Present evidence suggests that only living cultures, either virulent or avirulent, produce effective immunity.
3) There is a definite, but still poorly understood, relationship between silage feeding and listeric infection in ruminants.
4) The epidemiology of all forms of listeric infection remains poorly understood, but evidence is accumulating rapidly that non-clinical carriers, both animal and human, play a most important part in transmission of the disease.
5) The pathogenesis of the disease is not known. Infections resulting in disturbances of the central nervous system appear to have a different pathogenesis from those producing abortion or other disturbances of pregnancy.
6) Although *L. monocytogenes* usually grows well after isolation, it is often difficult to isolate from infected material. There is a real need for refinements in isolation techniques.
7) Public health laboratories should be encouraged to send "blind" cultures of *L. monocytogenes* to local laboratories for tests of proficiency in recognition of the bacterium.
8) Rabies diagnostic laboratories should be alert to the fact that when inoculated mice die within the first four postinoculation days, they should be cultured for *L. monocytogenes*.
9) *L. monocytogenes* cross reacts serologically with many other bacteria and this fact must be considered in the interpretation of serological tests.
10) Listeric infection in man is far more common than generally suspected. Well over 1,000 cases have been confirmed bacteriologically throughout the world. Listeriosis is most commonly manifested by meningitis in infants and in persons older than 40 years. In adults, listeriosis is often superimposed on some other potentially grave disorder. It may also produce interruptions of pregnancy.
11) The role of *L. monocytogenes* in the repeated abortion complex remains controversial, but there is sufficient evidence to warrant further investigation of this possibility.
12) *L. monocytogenes* may sometimes produce a syndrome simulating infectious mononucleosis but it is not involved in true infectious mononucleosis.
13) Listeric infection may follow administration of cortisone or its derivatives and the physician should be alert to this possibility.
14) Tetracycline is the antibiotic of choice in all listeric infections.

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