Histopathology of *Listeria monocytogenes* After Oral Feeding to Mice

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Ingestion by mice of *Listeria monocytogenes* in drinking water induced micro-abscesses of the liver and ulcerative enteritis. In pregnant mice, these lesions were larger and more extensive than in nonpregnant female and male mice. Pregnancy is interrupted by a purulent panmenitis and necrotizing placentitis. The early microabscess is unique in that it consists of monocytic cells with bizarre-shaped nuclei. The experimentally induced pathological changes in livers and intestines of mice used in this study are similar to those observed in listeric neonatal septicemia of human infants.

Oral infection with *Listeria monocytogenes* of several species of animals (mice, rabbits, sheep, and goats) has been reported (1), but description of the histopathological lesions is meager.

This report presents the histopathology found after oral ingestion of *L. monocytogenes* for 24 hr by male, female, and pregnant mice. A comparison is made of the histopathological lesions in mice and the lesions in human infants with so-called "granulomatosis infantiseptica" described by Reiss and his associates (9, 10).

**MATERIALS AND METHODS**

White Swiss mice (Albany strain) weighing 18 to 22 g were used. Strain no. 6053 of *L. monocytogenes*, type I [received from Thelma F. Muraschi of this Division in the lyophilized state and maintained on blood agar; identification and type determined by its somatic precipitinogens (8)], was inoculated into 200 ml of Tryptose phosphate broth and incubated for 18 hr at 36°C. Such cultures yielded 10^7 to 10^8 bacteria per ml. The broth culture was centrifuged and the sedimented microorganisms were resuspended in 100 ml of sterile water. Mice were denied water for 12 hr, and then oral feeding was instituted for 24 hr. Bacteria-laden water (40 ml) was provided for each group of five mice. The colony count of the water after 24 hr was 10^6 viable *L. monocytogenes* per ml.

Mice were sacrificed on the 4th, 7th, and 14th days after completion of 24 hr of oral feeding. Portions of each organ were placed in Bouin solution for fixation, and other portions were cultured on blood-agar and esculin agar (5). Sections of tissues were stained by the hematoxylin-eosin technique and by the Brown-Brenn method (2) for demonstration of gram-positive bacteria.

**RESULTS**

No gross evidence of lesions was apparent, but, on microscopic examination, definite and characteristic lesions of *L. monocytogenes* infection were seen.

Liver lesions in all mice were found on the 7th day after oral feeding. Surprisingly, on the 14th day there was little evidence in male and non-pregnant mice of the lesions noted on the 7th day; however, in pregnant mice the lesions were more numerous and larger on the 14th day.

The characteristic hepatic lesion was a micronodular lymphoma surrounded by normal cords of hepatic cells (Fig. 1), and consisted of an accumulation of monocytes. The cytoplasm stained faintly and the cell borders were indistinct. The nuclei were irregular shapes with pear, club, and hook-like forms (Fig. 2). Accumulation of these cells was seen both early and late around the interlobular vein. With the onset of necrosis, polymorphonuclear leukocytes appeared in the lesions. Some lesions became quite large without necrosis or granulocytic infiltration. The lesions remained discrete and did not coalesce.

**Gastrointestinal tract.** Lesions of the intestine were second in frequency. The earliest lesion at the 7th day was a submucosal infiltration of monocytic cells in the small intestine (Fig. 3). By the 14th day, all mice (male, female, and those pregnant) showed a mild to severe focal ulcerative enteritis with sloughing of mucosal cells into the lumen of the intestine. Lesions of the liver were sometimes noted before any appreciable intestinal lesions appeared.

**Lungs.** Lungs were the third most regularly involved organ in the experimentally induced listeriosis. An interstitial pneumonitis of varying severity was seen in 60% of the male and female mice. The inflammatory cells again were the monocytes with bizarre nuclei.

**Uterus and placenta.** The uterus was not...
infected in nonpregnant mice. Six of ten pregnant mice, however, had interruption of pregnancy with fetal death. We observed diffuse purulent mononexitis and necrotizing placentitis similar to that described in pregnant rabbits with listeric infection (7). The fetal infection was the probable cause of death.

Although it was difficult to demonstrate gram-

Fig. 1. Microabscesses of liver of mouse. X150.

Fig. 2. Microabscess of liver of mouse. Bizarre nuclear forms. X1,620.

Fig. 3. Early enteritis of mouse. X150.

Fig. 4. Microabscess of human liver (infant). Courtesy of H. J. Reiss. X225.

positive L. monocytogenes in the hepatic or intestinal lesions, microorganisms were seen in the necrotic placental debris.

Other viscera. A lymphoid hyperplasia of the
spleen was commonly seen by the 14th day. No lesions were found in the heart, kidney, adrenal, or pancreas.

Isolation of L. monocytogenes from viscera. L. monocytogenes was recovered from the livers of three of five male mice on the 4th day after oral feeding and from the lung of one mouse. On the 7th day, only one female mouse yielded positive cultures of the test microorganism from the liver, lung, kidney, and spleen. At the 14th day, cultures of all organs from all mice were sterile. Cultures of cardiac blood were sterile at all times in all mice.

DISCUSSION

These observations indicate that oral ingestion of L. monocytogenes by mice can induce an infection characterized by microabscesses of the liver and ulcerative enteritis. The lesions are more severe and extensive in pregnant mice in which a purulent panmetritis and necrotizing placentitis occur.

In an extensive review of the literature of listeriosis, Gray and Killinger (4) presented ample evidence that the principal portal of entry of the etiological agent is the mouth. Fetal listeriosis in pregnant women has been associated with ingestion of L. monocytogenes-contaminated milk from infected cows. Dijkstra (3) demonstrated a carrier state in the intestinal flora of cattle in Holland. Bojen-Møller and Jessen (1) found that the feces of man, particularly workers in slaughter houses, harbor L. monocytogenes. Thus, feces may be a source of dissemination.

There is still another possible route of infection of the female. Our earlier experiments (6) with female rabbits suggest that intravaginal infection can induce listeric vaginitis and focal endometritis leading to interruption of pregnancy. L. monocytogenes does not survive pH 4.5, the hydrogen ion concentration of normal vaginal secretions of rabbits. Bleeding from menses or exudate of cervicitis will buffer the vaginal secretions to pH 7.2. If the vaginal secretions in rabbits are maintained at a pH of 7.2 by daily douching with phosphate-buffered saline, listeric vaginitis can be induced in nonpregnant rabbits. The infection extends upward by way of the lamina propria into the cervix and endometrium. Thus, in the epidemiology of listeriosis, there are at least two routes of possible infection of the pregnant female, oral and intravaginal.

The original articles by Reiss and his associates (9, 10) on listeriosis of the newborn in East Germany, "granulomatosis infantiseptica," include a review of 24 reported cases and their own 5 cases. Twenty-eight cases showed micro-abscesses of the liver (Fig. 4), with eight cases presenting accompanying enteritis (Fig. 5). Other infected organs include the esophagus (Fig. 6), stomach, kidney, spleen, lung, bronchi, pharynx, tonsil, thyroid, skin, and lymph nodes. In infants, a generalized septicemic infection apparently occurs.

Later, Sepp and Roy (11) reported 11 cases of neonatal L. monocytogenes septicemia in Canada. In eight autopsied cases, they found the following
distribution of involvement: central nervous system (meningitis), eight; adrenals, eight; lungs, eight; liver, seven; gastrointestinal tract, six; spleen, four; kidney, three; bone marrow, three; pancreas, two; thymus, two; urinary bladder, one; peribronchial nodes, one. In one case, the placenta had listerial lesions. Included also was a recovered case of tonsillitis and pharyngitis in a child from whose pharyngeal secretions \textit{L. monocytogenes} was isolated. The authors described the characteristic monocytic infiltrate with bizarre nuclear forms. With necrosis, polymorphonuclear leukocytes appeared in the lesions. Although not consistent, the cells in the cerebrospinal fluid from several of their cases of meningitis were predominantly monocytic; in other cases granulocytes were predominant, possibly from older lesions.

The lesions of the liver and intestine of mice described by us are similar to those found by Reiss and associates (9, 10) and by Sepp and Roy (11) in infants.

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