the glycogen deposition assay. 6-Methyl-Δ^1-F, like Δ^1-F, does not induce sodium and water retention on the assay for electrolyte activity, but in contrast to Δ^1-F, this steroid acts as a diuretic and natriuretic agent.

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Received November 14, 1956. P.S.E.B.M., 1957, v94.

Reaction of Splenic Tissue in Culture to *Listeria monocytogenes*. (22886)

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(Introduced by C. K. Whitehair)
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A study of *Listeria monocytogenes* in tissue culture was conducted because of the peculiar behavior and wide host susceptibility in experimental animals(1,2) as well as in natural hosts(3-8). Listeric infections in rabbits cause a monocytosis with a generalized infection while ruminants usually manifest a severe encephalitis. Abortions due to *L. monocytogenes* have been reported in cattle, sheep, goats, swine and rabbits(9,7). In recent years several reports of abortion, and early deaths in infants due to *L. monocytogenes* have come from both sectors of Germany(10-12). The disease has been called *granulomatosis infantiseptica* and is characterized by a granulomatous reaction associated with capillary endothelium(10,13). Gray *et al.*(14) reported that maceration of tissues followed by refrigeration increased the probability of recovery of the organism from infected tissues; other workers have reported similar results. Because of this strange behavior inoculated tissue cultures were studied for this same phenomenon.

Materials and methods. The culture of *L. monocytogenes* used was isolated from the brain of a calf with listeric encephalitis. Previously, Gray *et al.*(16) reported that the concentration of bacteria obtained from a suspension made to match the 0.5 tube of a MacFarland nephelometer was adequate to produce symptoms and lesions typical of *L. monocytogenes* infection in rabbits. This concentration of bacteria did not suppress growth of tissue in culture. The bacterial suspension was prepared by washing the 24-hour growth off tryptose agar (Difco) slant with sterile saline. This suspension was diluted with saline until the density matched the 0.5 tube of a MacFarland nephelometer when read in a Cenco Sheard Sanford photelometer. An inoculum of 0.01 ml of this bacterial suspension was added to the tissue cultures from tip of 27 gauge needle. The double coverslip method of Maximow(15) was used throughout. Explants of adult rab-

* Partial fulfillment for degree of Doctor of Philosophy, Michigan State University, East Lansing. Published with approval of Director of Mich. Agri. Exp. Station as Journal Article No. 1986.

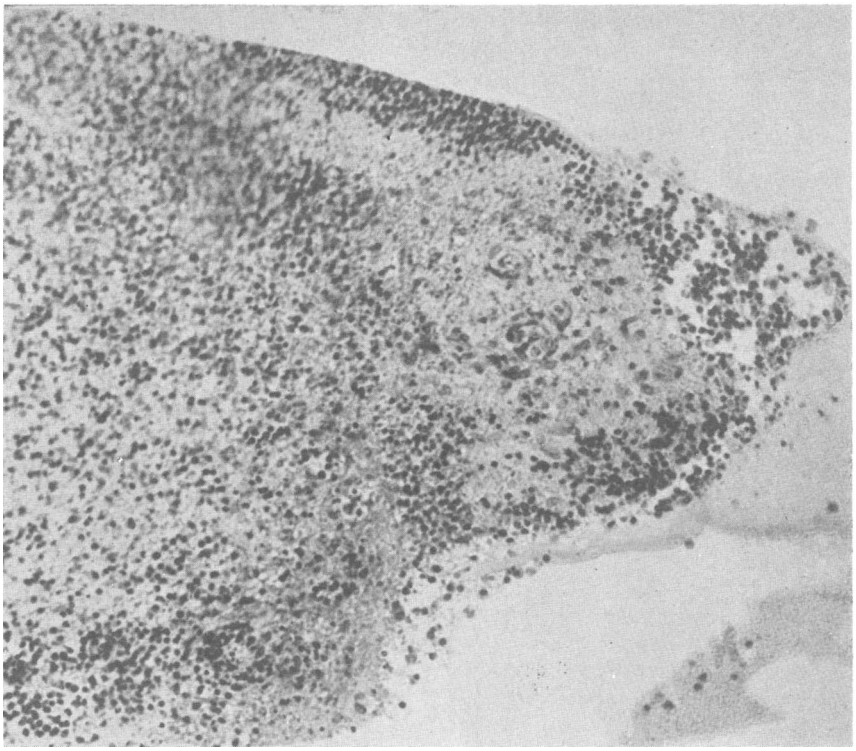

FIG. 1. Inoculated culture after 24 hr incubation. Note beginning of proliferation of vascular endothelium.

bit spleen were placed in a plasma clot and the nutrient medium consisting of 60% solution 199, 20% embryo extract and 20% rabbit serum was added. The cultures were incubated as a lying drop at 37°C. All cultures were examined daily for cell migration and outgrowth. Uninoculated controls were compared with cultures which had been inoculated with the bacterial suspension. For histopathological studies, inoculated and control cultures were removed daily and fixed in Bouin's fluid. Serial sections were made of the entire explant and outgrowth, stained with hematoxylin-eosin, and Weigert-Van Gieson for connective tissue determination. To test the viability of *L. monocytogenes* in tissue culture, the inoculated explants were removed from culture slides, placed in tryptose broth and incubated at 37°C. To study the effect of refrigeration on exposed explants, some tissue cultures were held at 4.0°C while still in Maximow slides according to the following schedule. After 4 days at 37°C, 6 tissue cultures were removed from incubator each day for 4 consecutive days and placed in refrigerator (4.0°C) for periods of 7, 16, 23, and 31 days. Then each group of 6 cultures was unsealed, placed in tryptose broth and incubated at 37°C for at least 2 weeks.

Results. Living cultures. During the first 24 to 40 hours following incubation, polymorphonuclear leucocytes migrated outward from the explant, gradually became inactive and degenerated. After 2 days large, ameboid, phagocytic cells were seen to move slowly in a random manner. Bacteria were distributed diffusely throughout the cultures.

Fixed cultures. Explants which were removed daily for 6 consecutive days and sectioned serially revealed definite pathological changes. The reaction began by what appeared to be a proliferation of the vascular sinus endothelium. As early as 24 hours, two layers of endothelial cells were seen circumscribing a small vessel. Radiating from this blood vessel were single rows of cells infiltrating the surrounding tissue (Fig. 1). The nuclei of these cells were vesicular, elongated

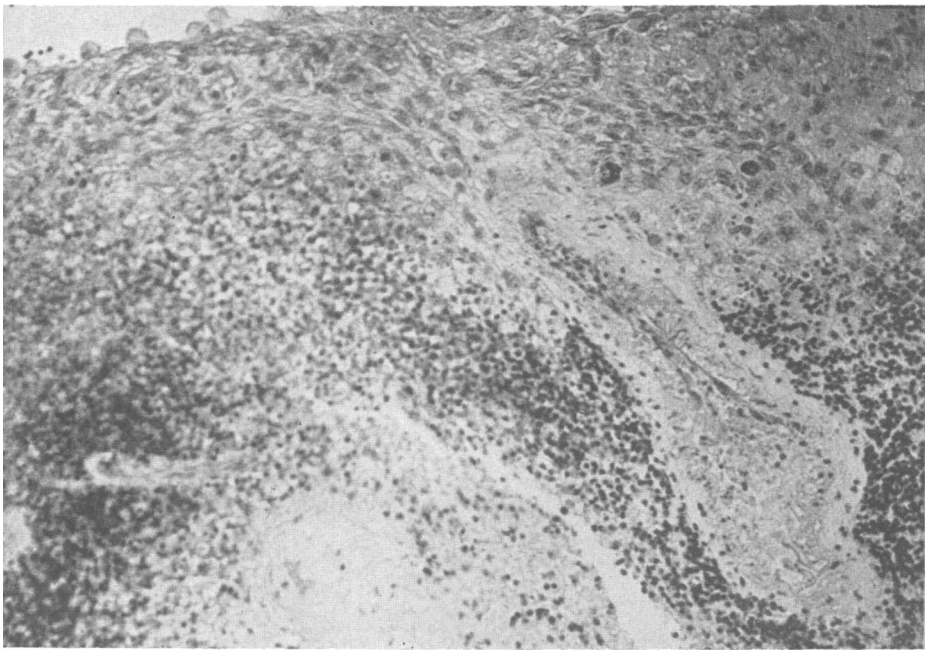

FIG. 2. Inoculated culture after 6 days incubation. Note formation of granuloma as a result of cellular proliferation.

and pleomorphic. As a result of the continued proliferation of the endothelium, the cells appeared as swirls around the vessels until the involved area was of considerable size at the end of the 6-day period and took on the characteristics of a granuloma (Fig. 2). The epithelioid cells which made up the granuloma, or cell mass, always circumscribed a vascular sinus. Sections stained with Weigert-Van Gieson connective tissue stain revealed elastic fibers around the lumen of the vessels in the center of the proliferated cells. Exceptionally large, round macrophages were seen at the margin of this cell mass after at least 4 days. They appeared to be identical with the cells of the granuloma, but were more spherical and usually contained a yellow pigment. Attempts to associate the presence of the bacterium with this pigment failed.

Uninoculated control cultures did not exhibit any proliferative cellular reaction. There was some necrosis at the center of the explant due to the inability of the nutrients to penetrate the explant (Fig. 3).

Refrigeration. When a 24-hour explant was unsealed, and placed in tryptose broth, *L. monocytogenes* grew luxuriously after 18 hours incubation. However, when exposed explants were incubated 48 hours or longer and then cultured there was no evidence of bacterial growth even after 1 week incubation at 37°C. Nevertheless, if these exposed explants were placed in the refrigerator for periods of 7, 16, 23, and 31 days, *L. monocytogenes* was recovered from broth cultures after 48 hours of incubation at 37°C.

Discussion. Histopathological study revealed a reaction definitely associated with the vascular endothelium. It is questionable that these cells are true monocytes, but they have characteristics which are associated with mononuclear phagocytes. Also, it should be noted that *L. monocytogenes* causes a very severe monocytosis in rabbits. It has been established that the vascular sinus endothelium has been considered a part of the reticulo-endothelial system. However, considerable controversy remains regarding the origin of the mononuclear phagocyte. Forbus (17) stated that the mononuclear wandering cells are derived either from primitive mesenchymal cells or from vascular sinus endothelium, with the latter being the theory most widely accepted. It is of interest that the

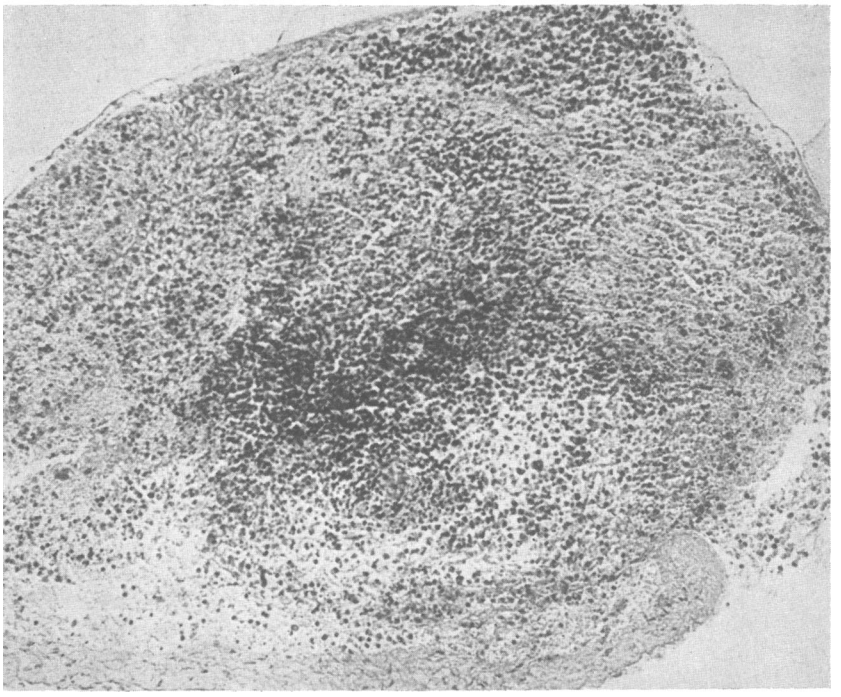

FIG. 3. Control culture after 4 days incubation. No proliferative cellular reaction.

granuloma formation is similar to that reported by German workers(4,10,13) describing the pathology associated with *granulomatosis infantiseptica*. It is their belief that the granuloma is derived from vascular sinus endothelium.

It is possible only to speculate on the phenomenon of increasing recovery of *L. monocytogenes* following a period of refrigeration. Perhaps some inhibitory substance acts as a bacteriostatic agent at 37°C, but when refrigerated, no longer retains this bacteriostatic property. Another possibility is that some biological system may become inactivated at 4°C allowing the bacteria to grow on artificial media.

Summary. Serial sections of infected tissue cultures revealed a granuloma due to the proliferation of the vascular sinus endothelium. This reaction was similar to that reported by German authors in describing lesions of *granulomatosis infantiseptica*, a disease caused by *L. monocytogenes*. Infected tissue cultures incubated 48 hours or longer required refrigeration for at least 7 days in order to demonstrate the presence of *L. monocytogenes*.

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Received November 15, 1956. P.S.E.B.M., 1957, v94.

Mitotic Activity of Mast Cells. (22887)

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Michels(1) in his review of mast cells comments "practically without exception investigators who have encountered mitotic dividing mast cells have stated that instances of them are extremely rare." This opinion has apparently not changed in recent years judging by comments made by Asboe-Hansen(2) at Josiah Macy, Jr. Foundation Conference on connective tissues. On the other hand mast cells are often seen in what appear to be isogenous groups as shown here in Fig. 7 and it has been assumed(3) that mitosis or amitosis has occurred. Direct evidence has been lacking, however. That cell division has been observed rarely in mast cells does not necessarily mean that it does not occur even with considerable frequency. Observations may have been made at the wrong time or under unfavorable conditions or the division figures overlooked. As usually stained with toluidine blue, nuclear material is either obscured by heavy granulation or is relatively colorless if the stain has been acidified. This latter procedure facilitates the identification of mast cells but greatly reduces the chances of seeing mitotic figures.

In the present study, a considerable number of mitotic figures were observed in mast cells of the rat following injection of the histamine liberating compound 48/80.

Materials and methods. A Long-Evans strain of male rats 100 days old or more was used. Animals were injected intraperitoneally with the histamine liberator, compound 48/80.* Rats in Group 1 were given 100 γ daily (concentration of 1000 γ/ml of saline) for 7 days and then were killed either on 8th day or in a few cases on 16th or 24th day. Those in Group 2 had 500 to 1000 γ daily for the same length of time and were killed on the 8th day. At time of death preparations of mesentery and subcutaneous films of connective tissue of the back were made by stretching the tissue over Bristol board rings with punched openings about 15 mm in diameter. Preparations were fixed in cold formol and absolute ethanol (10:90) and stained in 0.1% toluidine blue which in most cases was not acidified. After dehydration and clearing, the tissue was cut from the ring and mounted.

Observations. In Group 1 (with the smaller dosage) the 48/80 was sufficient to result in disappearance of the large-sized, heavily granulated mast cells usually present in the mesentery. There remained, however, a considerable although variable number of medium-sized cells having diameters of 14 to 24 μ with fewer granules. Among these cells mitotic figures could be readily observed (Fig. 2 to 5).

It is difficult to give an accurate quantitative count of the mast cells in division because the mitotic figures are relatively widely and unevenly spaced and are easily overlooked. In one preparation of mesentery, however, covering approximately 12 square mm. 6 mitotic figures were seen among a total of 364 mast cells. In another preparation 2 mitoses were seen in one high power field. In other preparations dividing cells were more widely spaced but could be found with careful searching.

* The compound 48/80 was kindly supplied by Dr. E. J. deBeer of Wellcome Research Laboratories.