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The Histopathology of Experimentally Infected Hamsters with the Lyme Disease Spirochete, *Borrelia burgdorferi* (42251)

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Abstract. Seven hamsters, experimentally infected with *Borrelia burgdorferi,* were examined by both cultural and histological techniques at 1 to 9 months postinfection. Spirochetes were detected in the spleen, kidney, or eye of all animals by culture and in the spleen, kidney, eye, liver, or heart blood of five of seven animals by histological examination. Two animals showed nonspecific hepatic portal lymphocytic infiltration, while five of the hamsters displayed no significant histologic signs of inflammation or granuloma formation in the major organ systems. Synovitis and arthropathy did not occur. All animals showed some degree of follicular lymphoid hyperplasia of the spleen. Spirochetes were predominantly extracellular with a rare organism appearing to be partially within a macrophage.

Lyme disease, a systemic human illness, was reported in 1977 by Steere *et al.,* following recognition of a cluster of cases in Connecticut (1). In Europe the disease was called erythema chronicum migrans (2–4), achrodermatitis chronica atrophicans (5), and Bannwarth's syndrome (6). These disorders are now recognized as components within the spectrum of Lyme disease (7). The syndrome, caused by a newly described tick-borne spirochete, *Borrelia burgdorferi* (9), begins as a rash (erythema chronicum migrans) (10) at the site of the tick bite, with subsequent organ involvement by hematogenous dissemination in some patients (11, 12). Involvement of the central nervous system (13), heart (14), and joint synovia (1) occurs in humans at least partially due to the direct presence of spirochetes in these sites. Deer act as a reservoir host for spirochete-infested ticks (*Ixodes dammini, I. pacificus*) during the winter (15), with subsequent involvement of small animals during the late spring and summer (16).

Recent observations have delineated the histopathologic changes seen in human Lyme arthritis (17), human myocarditis (18), and necrotizing chorioretinitis (8). While *B. burgdorferi* has been found in the blood of infected animals (16, 19), data on the pathogenesis of the organism within animal hosts is lacking. Information on the extent, if any, of organ damage in animal hosts is also lacking. We report our findings on the histopathology of, and demonstration of spirochetes in, Syrian hamsters experimentally infected with *B. burgdorferi.*

Materials and Methods. A total of seven (four males, three females) 5- to 10-week-old Syrian hamsters (Engle Laboratories, Farmington, Ind.) were inoculated intraperitoneally with approximately 10^8 cells of *B. burgdorferi.* The spirochetes (TLO-030, human spinal fluid isolates) were supplied by Allen Steere, M.D., Yale University School of Medicine, New Haven, Connecticut. They had been maintained in BSK (modified Kelly) medium (20) with 0.1–0.2% agarose (Seakem KE, FMC Corp., Rockland, Me.). The organisms were unwashed, suspended in modified Kelly culture medium, and passed *in vitro* no more than three times. Hamster blood and organs were cultured as previously described (21). Indirect immunofluorescence using immune rabbit serum against *B. burgdorferi* was used to identify isolates. Cultures were examined by darkfield microscopy at weekly intervals for the presence of spirochetes. The animals were fed a standard chow and observed for illness for up to 9 months postinoculation, and sacrificed for histologic examination in a range from 1 to 9 months postinfection. At

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necropsy all organs including the major limb joints (after HCl, triacetic acid, Na^+-K^+ tartrate decalcification) were processed for 10% buffered Formalin-fixed, paraffin-embedded tissue sections using the Fisher histomatic tissue processor (266 MP), and stained with H & E and Mallory trichrome stains. The Mallory trichrome stain had previously proven to be useful in histologically differentiating eosinophilic collagen from deposition of eosinophilic fibrin within the damaged joint synovia of human Lyme arthritis patients (17), and was employed routinely in this study. Slide imprints of the liver and spleen were made from the cut surfaces of the fresh organs and stained with H & E. All sections including the tissue imprint (touch preparation) slides were stained for spirochetes using a modification of the Dieterle silver-impregnation method (22). Control slides consisted of pellets of spirochetes from maintenance cultures of *B. burgdorferi.*

Results. At necropsy all animals were well nourished with normal appearing hair coats and no limb joint swelling. No abnormal fluid was found in the body cavities including the pericardial sac. Orthopedic joints, synovia, tendons, and associated musculature were unremarkable and gross abnormalities were not seen in the thoracic and abdominal viscera.

None of the animals displayed significant histologic abnormalities in the brain, endocrine system, lungs, heart, pancreas, stomach, jejunum, genitourinary system, lymph nodes, and synovia. Whole eye sections showed occasional mononuclear phagocytic cells in the ocular vitreous fluid of animals 3 and 4. All animals showed some degree of follicular lymphoid hyperplasia of the spleen (Fig. 1). Lymphocytic infiltration of occasional hepatic portal triads without cholestasis or cholangitis, were present in animal 4, while a rare lymphohistiocytic aggregate was seen in the hepatic parenchyma of animal 3 (Fig. 2). Other than these no areas of inflammation, granulomatous formation, or tissue necrosis were seen in any animal. Blood vessels appeared normal throughout.

Table I lists the sites of premorbid spirochete isolation by culture and the organs where spi-

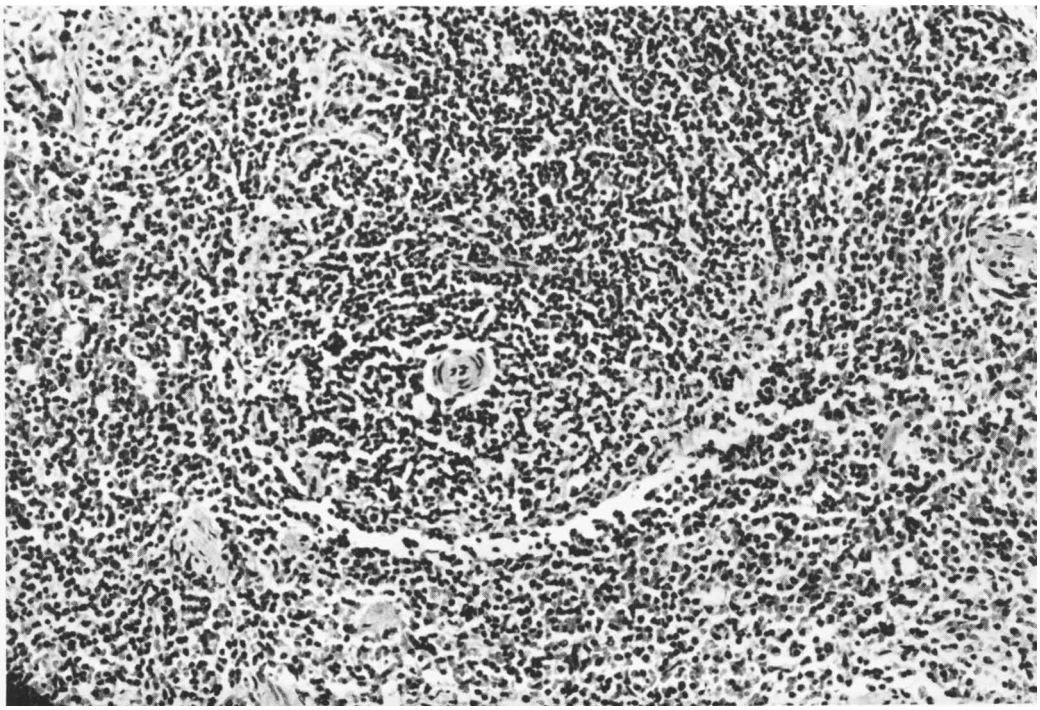

FIG. 1. Hyperplastic white pulp (lymphocytic follicle) was present in all spleens. H & E ×40.

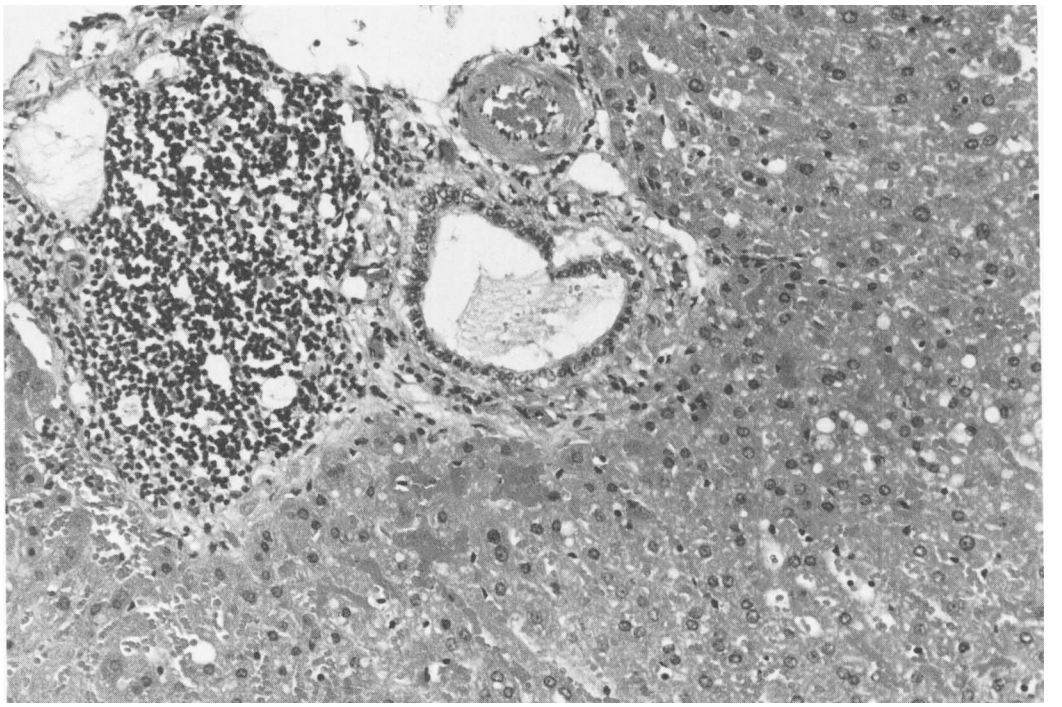

FIG. 2. Lymphocytic portal triaditis in animal 4. H & E ×40.

rochetes could be seen in postmortem tissue sections. The Dieterle stain demonstrated the spirochetes by a jet-black silver precipitate against a yellow background similar to other silver impregnation methods for treponemes. The lengths and morphology were comparable to the controls (Fig. 3). Spirochetes were particularly easy to visualize in the vitreous of the eyes of animals 4 and KI-I. Here the morphology of the organisms was strikingly similar

TABLE I. CORRELATION OF SPIROCHETE CULTURE RECOVERY AND TISSUE VISUALIZATION

Animal	Months from inoculation to necropsy	Culture positive for spirochetes (14 days after inoculation)	Spirochetes seen in tissue sections	Pathologic tissue changes[a]
1	1	Spleen, kidney	None	None
2	1	Spleen, kidney	Liver/spleen imprints	None
3	1	Spleen, kidney, eye	Kidney	Rare lymphocytic focus in liver
4	7	Spleen, kidney, eye	Spleen, kidney, eye, heart blood	Chronic portal triaditis
KI-1	8	Spleen, kidney	Kidney, eye	None
KI-3	9	Spleen, kidney, eye	None	None
KI-4	6.5	Spleen, kidney, eye	Liver, spleen, kidney, heart blood	None

[a] All had varying degrees of splenic lymphoid follicle hyperplasia.

to that seen in the maintenance culture controls (Fig. 3). Ocular spirochetes were in greater numbers in the vitreous relative to the numbers seen in the liver, spleen, or kidneys. Liver–spleen slide imprints revealed spirochetes in only one animal. Aspiration cultures of the spleen and kidney yielded spirochetes in six of the seven hamsters. Spirochetes were seen in the red pulp of the spleen and the renal tubules of the kidneys. None were found in the renal glomeruli. Figures 3–5 are illustrative of the organisms seen as listed in Table I. Spirochetes were in very low numbers, occurring singly, necessitating the examination of many oil-immersion fields (×1000) to adequately demonstrate them.

Morphology varied from relatively short and straight forms (like a long bacillus) to very long, undulating forms with a characteristic hook at one end. "Hairpin" shapes were also not uncommon. Lengths and widths fell within the range of 4–39 × 0.19–0.25 μm as previously described (16, 23). Dieterle silver staining, which depends on the reduction of the metal, varied from uniform to granular. Spirochetes were predominantly extracellular, with a rare organism appearing to be partially within a macrophage.

Discussion. The inoculation of significant numbers of the Lyme disease spirochete, *B. burgdorferi,* intraperitoneally into these seven hamsters did not induce significant histopathologic changes in any organ save for mild splenic lymphocyte follicular hyperplasia in all and intermittent chronic portal triaditis in two animals. The lymphocytic aggregates in the livers of animals 3 and 4 were focal in distribution, and spirochetes were not seen directly within them. Thus, while organ infection was documented by both culture recovery and direct tissue visualization, the presence of spirochetes in hamster tissues did not elicit an appreciable and constant inflammatory infiltrate. While a humoral response to Lyme spirochete infection has been shown to occur in animal hosts (16), an inflammatory cell response within infected animal tissues appears limited if this group of hamsters reflects the situation in tick-infested animals in Lyme disease endemic geographic areas. The lymphocytic infiltrates in the livers of two hamsters may also be coincidence since no histopath-

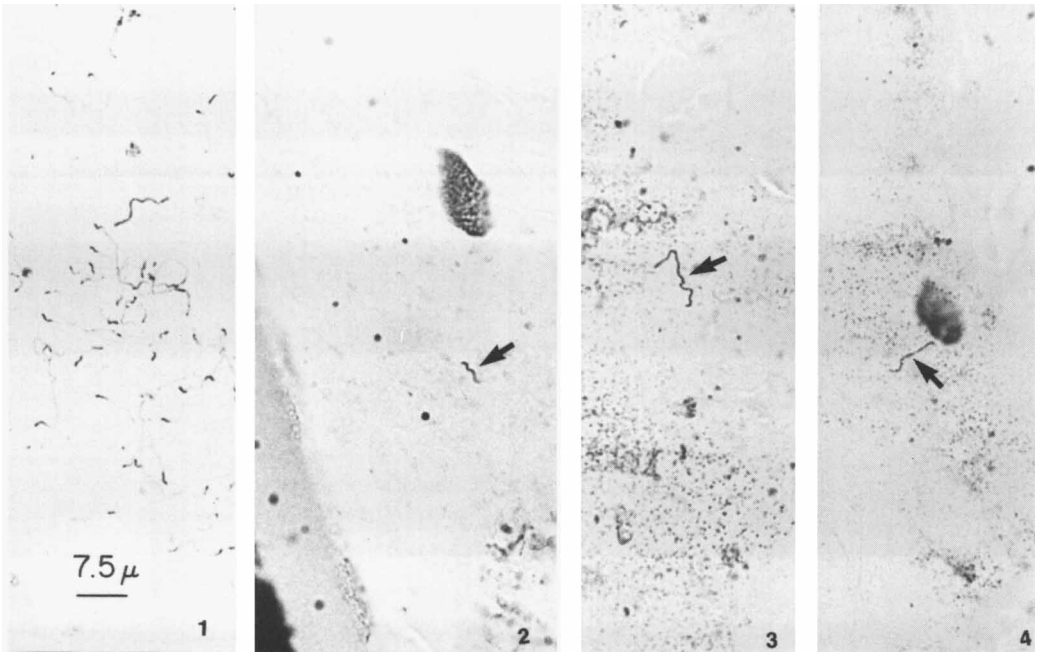

FIG. 3. (1) *B. burgdorferi* maintenance culture control. (2–4) Spirochetes in vitreous fluid (whole mount eye sections) of two hamsters. Dieterle stain ×1000.

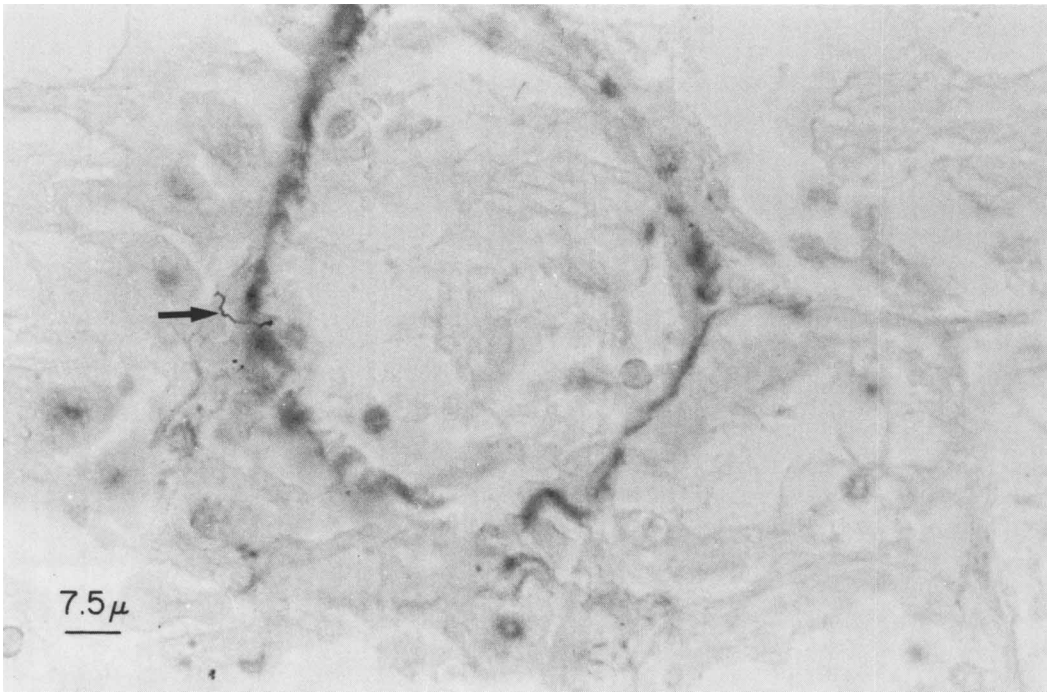

FIG. 4. Spirochete (arrow) in renal tubule. Dieterle stain ×1000.

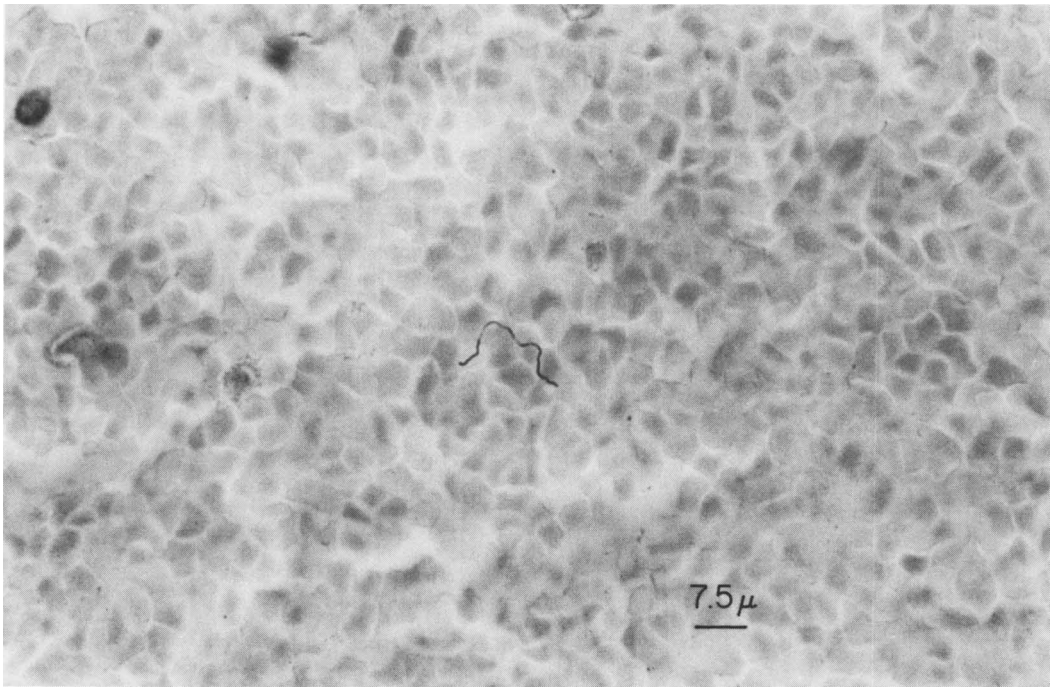

FIG. 5. Spirochete in heart blood. Compare the length with red cell diameter. Dieterle stain ×1000.

ologic alterations were seen in five of seven hamsters, with three of these same five having demonstrable spirochetes in tissue sections. Microangiopathic lesions (obliterative endarteritis) as seen in human Lyme arthritis (17) were never seen in any sections of these hamster tissues. The spirochetes' attraction for the eye may be related to the viscosity of the vitreous as well as the glucose and electrolyte content. Ocular vitreous viscosity qualitatively is similar to that provided by adding 0.1% agarose to BSK media.

Once infection occurs, *B. burgdorferi* appears to spread hematogenously and was recovered from the peripheral blood of hamsters in the first 6 days of infection, but not after 2 weeks (21). Thus we were surprised to find unequivocal spirochetes in the blood of the left ventricles of the heart 7 (animal 4, Fig. 5), and 6.5 (animal KI-4) months postinoculation. In addition to evidence that the spirochetes can persist in organs on a chronic basis, it appears that they also retain the capacity for spirochetemia on a relatively long-term basis. We do not know at the present time if the spirochetes multiplied *in vivo* during this period, or if they merely persisted. The persistence of *B. burgdorferi* in the natural environment may be a function of chronic intermittent spirochetemia.

The lack of morphologic organ damage and severe inflammation in these experimentally infected hamsters contrasts with histopathologic alterations seen in infected human tissue (17). We do not know if the preferred host for the immature forms of *I. dammini,* the white-footed mouse *Peromycus leucopus,* has minimal histopathologic organ damage when infected with *B. burgdorferi* paralleling the situation in the golden hamster. As many as 86% of the white-footed mice captured in endemic foci of Lyme disease had *B. burgdorferi* present in the blood, liver, spleen, kidney, or eye (24). While purely speculative, it is possible that the lack of an inflammatory cell response and intermittent spirochetemia in small animals may be factors in the continuing presence and maintenance of the spirochete in the natural habitat. While capable of causing disease in human organ systems, *B. burgdorferi* appears to have low virulence for some small animal hosts.

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