Lyme Arthritis

Spirochetes Found in Synovial Microangiopathic Lesions

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In 17 patients with Lyme disease, synovial specimens, obtained by synovectomy or needle biopsy, showed nonspecific villous hypertrophy, synovial cell hyperplasia, prominent microvasculature, lymphoplasmacellular infiltration, and sometimes lymphoid follicles. The larger surgically obtained specimens also showed striking deposition of fibrin in synovial stroma and a form of endarteritis obliterans. In 2 patients, spirochetes were seen in and around blood vessels by the Dieterle silver stain. Compared with 55 cases of other synovial disease, obliterative microvascular lesions were seen only in Lyme synovia, but marked stromal deposition of fibrin seemed nonspecific. These findings imply that the Lyme spirochete may survive for years in affected synovium and may be directly responsible for the microvascular injury. (Am J Pathol 1985, 118:26–34)

LYME DISEASE (LD) was recognized as a separate entity in 1975 because of close geographic clustering of children with what was thought to be juvenile rheumatoid arthritis (RA).[1] The illness is now known to be a complex multisystem disorder that affects both children and adults.[2] The disease often begins with a characteristic skin lesion, erythema chronicum migrans (Stage 1),[3] which may be followed by neurologic or cardiac abnormalities (Stage 2),[4,5] and is often followed by arthritis (Stage 3).[2,6,7] Joint involvement is typically intermittent and oligoarticular, but may become chronic, with erosion of cartilage and bone.[6,7] The disorder is caused by a newly recognized spirochete[8,9] that is transmitted by *Ixodes dammini* or related ixodid ticks.[10–12] It occurs in at least 14 states,[11,13] Europe,[14] and Australia.[15]

The histologic appearance of synovium in Lyme disease has been reported to be similar to that of rheumatoid arthritis.[2,6,7] Although the causative spirochete has been isolated from the blood, skin (erythema chronicum migrans), and cerebrospinal fluid of affected patients,[9] it has not yet been recovered from synovium. Similarly, the organism has been visualized in skin[16] or skin transudate specimens,[9] but not yet in synovium. Thus, it is not known whether the spirochete is alive in affected synovium. Alternatively, the synovial lesion might result from an indirect immune attack triggered by previous spirochetal infection.

We report here the histologic appearance of synovium in 17 patients with Lyme disease. In addition to the typical changes of other inflammatory arthritides, Lyme synovia often showed greater proliferative changes in arterioles, and spirochetes were seen near these vessels in 2 patients.

Materials and Methods

The 17 study patients met previously described clinical criteria for Lyme arthritis,[2,17] and all had elevated

Supported in part by the American Cancer Society, Regular Clinical Fellowship 5492A; U.S. Public Health Service Grants AM-20358, AM-07107, RR-00125, AI-17555, and AI-12211; and the Arthritis Foundation and its Connecticut Chapter.

Accepted for publication July 25, 1984.

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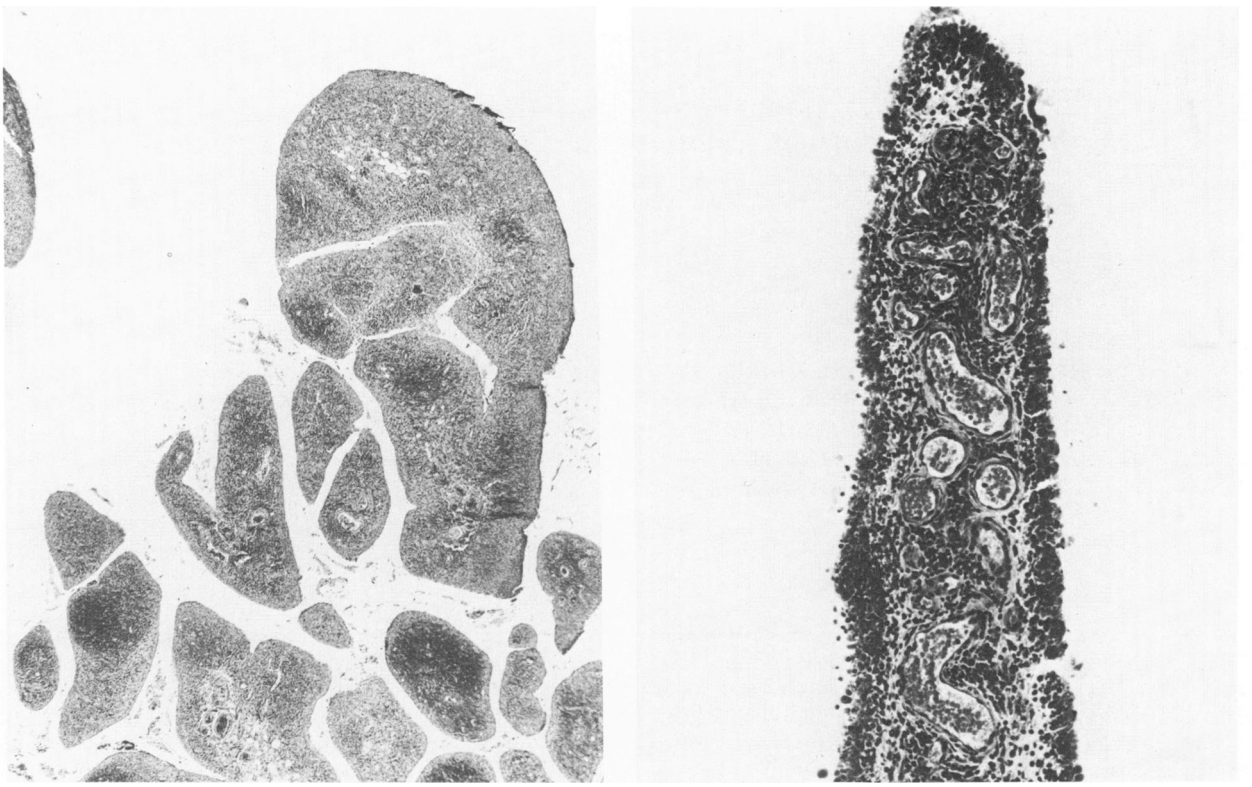

Figure 1A — Giemsa-stained surgical synovectomy specimen from a Lyme disease patient. (×20) **B** — Closed transcutaneous biopsy in a young patient with Lyme arthritis. (×40) Both show marked lymphoplasmacellular infiltrate. Notice the marked villus hypertrophy in the surgical synovectomy specimen (**A**). Lymphoid follicles with poorly delineated germinal centers are seen. None of these features are specific for Lyme arthritis, and may also occur in rheumatoid arthritis.

IgG antibody titers to the Lyme spirochete (>200 units, determined by ELISA).[17] Their ages ranged from 11 to 57 years (mean 36). Twelve were male, and 5 were female. Early in the illness, joint involvement was characteristically intermittent and oligoarticular, but became chronic (>6 months) in the knees in 14 of the patients. From 1976 to 1983, open surgical or arthroscopic synovectomies were done as therapeutic measures in 8 of the patients with chronic arthritis. In 1983 closed biopsies, performed with a 14-gauge Parker–Pierson needle, were done in the remaining 9 patients. They participated in a study of intravenous penicillin for the treatment of Lyme arthritis. The mean duration from the onset of arthritis to the time of synovectomy or synovial biopsy was 27 months (range, 3–84 months). All patients were receiving nonsteroidal antiinflammatory agents at the time of excision or biopsy, but none had received intraarticular steroid injections during the prior month.

Synovial samples were fixed in 10% neutral buffered formalin and in some cases, Helly's fixative (100 ml Zenker's solution plus 5 ml 40% formaldehyde). All samples were stained with hematoxylin and eosin (H&E), and larger samples, those from surgical synovectomies, were also stained with Putt's fibrin stain, Mallory's trichrome stain, periodic acid–Schiff (PAS) with and without diastase, elastic tissue stain (elastin, Verhoef–van Gieson), reticulin stain (Foote's), and Congo red and by the Dieterle silver impregnation method.

In order to eliminate observer bias, two pathologists (Y.J. and P.D.) studied blindly the histology of the 17 Lyme synovia along with 55 other synovectomy specimens from other synovial diseases. Histopathologic features searched for included synovial lining cell hyperplasia and hypertrophy; synovial villous hypertrophy; palisading necrobiotic granulomas and other forms of granulomatous inflammation; vascular changes, such as angiogenesis and vasculitis; vessel wall thickening; hemosiderin deposition; hemorrhage; sclerosis; necrosis; lymphoid follicles; and the nature of and predominant type of inflammatory cell in each specimen. Each of these features was scored as + (slight), ++ (moderate), or +++ (severe), or 0 if absent. The histopathologic findings were then correlated with the clinical information. Neither reader had any knowledge of the

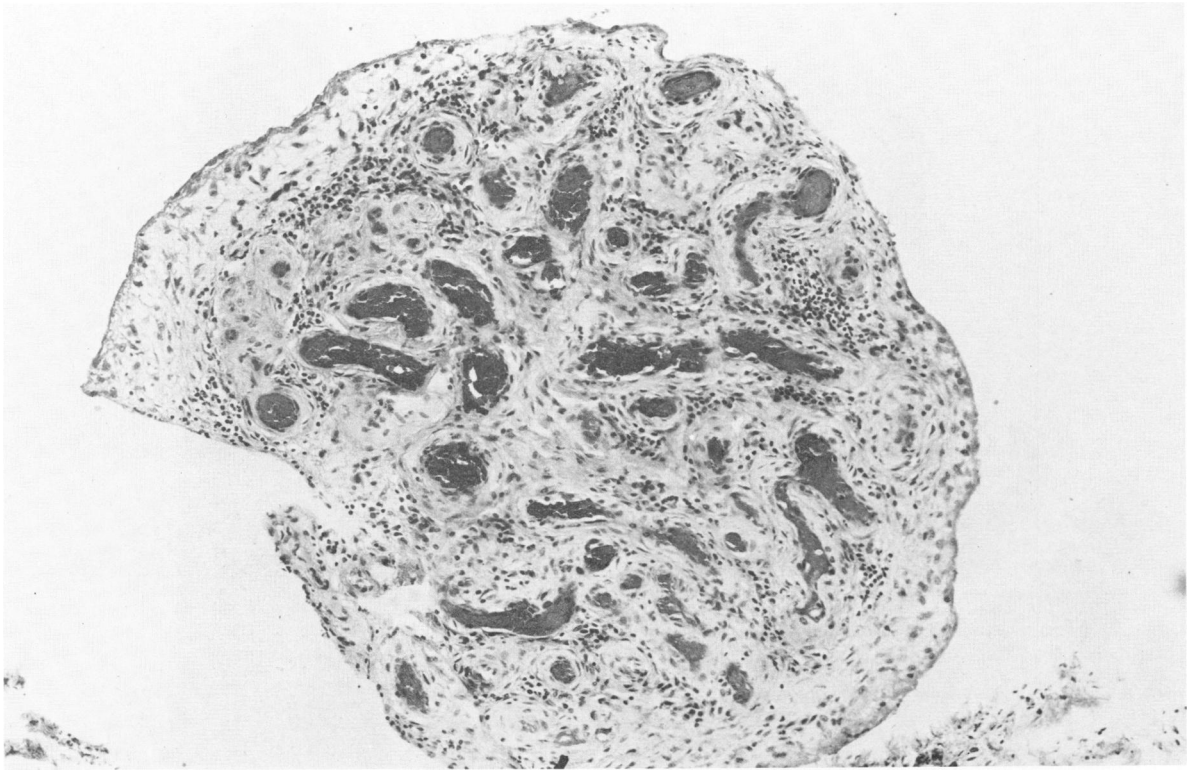

Figure 2—Cross-section of a Lyme arthritis synovial villus with accentuated and congested microvasculature. (H&E, ×40)

official diagnosis, the clinical information, or the observations of the other observer.

Results

Histopathologic Findings (H&E Stain)

The 17 synovial samples from Lyme disease patients showed a spectrum of pathologic findings, which depended on the amount of tissue available for examination. All Lyme cases had a moderate to marked chronic cellular infiltrate (Figure 1). In 14 of 17 samples, lymphocytes and plasma cells were very numerous. Lymphocytes were slightly predominant in 10; and plasma cells, in 4. In 6 of these cases, lymphoid follicles were present, but they had poorly delineated germinal centers. Neutrophils were the predominant inflammatory cell in only 3 samples, and eosinophils were absent in all. The 9 samples obtained by closed transcutaneous needle biopsies yielded tissue ranging from one fragment of synovium to a few small fragments of synovial villi. These demonstrated only chronic inflammatory cell infiltrates and nonspecific synovial lining cell hyperplasia.

The vasculature was prominent in 14 of the 17 cases. This prominence ranged from capillary arborization of the type seen in exuberant granulation tissue (Figure 2) to vascular dilatation with congestion, reminiscent of a lobular capillary hemangioma. Vascular microangiopathy, which resembled lupus "onion-skinning" in spleens, was seen in 5 cases. The onion-skin effect was produced by arteriolar muscle cell proliferation and concentric adventitial fibroplasia. By this process, complete luminal obliteration of arterioles was found in some patients (Figure 3). Lymphocytes were only rarely seen in the vascular walls, and luminal microthrombi were not demonstrated. Hemosiderin deposits were easily found in 87% of the surgically excised Lyme specimens, mainly near areas of capillary proliferation and vascular thickening. Numerous nondegranulated mast cells were observed in and near areas of vascular proliferation.

The 8 surgically excised specimens showed striking villus hypertrophy and acidophilic proteinaceous material, which often comprised about 50% of the total tissue volume (Figure 4). In 4 cases, this acidophilic material replaced entire synovial villi. This alteration was not true necrosis, because intact synovial cells could intermittently be seen lining the acidophilic material, suggesting that the proteinlike deposition occurred within villi and not solely on the surface. In addition, fibroblasts and other mononuclear cells were visible

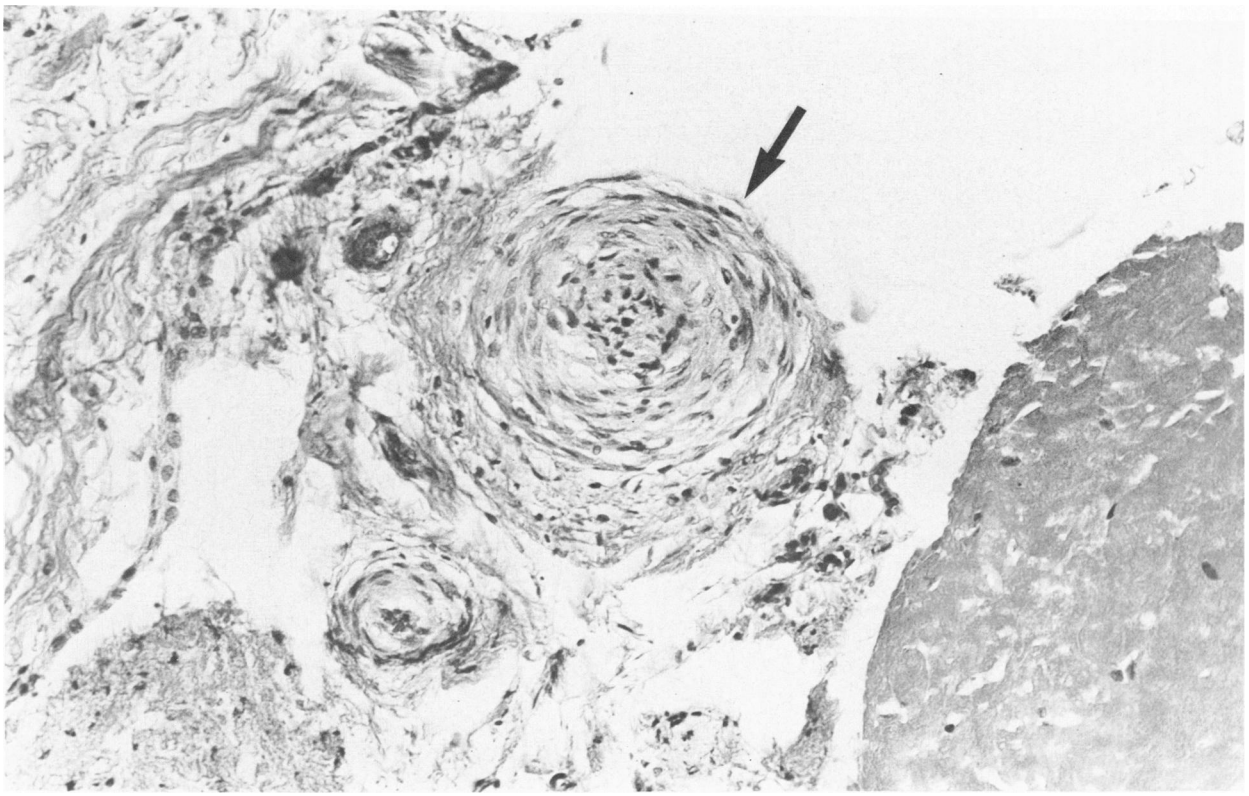

Figure 3—Obliterative microangiopathy in Lyme disease synovia ("onion-skinning"), similar to the obliterative endarteritis of syphilis. Note fibrinaceous stroma with intact mononuclear cells in lower right-hand corner. (Mallory trichrome stain, ×200)

within the matrix. Unlike detritus, this proteinaceous material was not birefringent by polarizing microscopy. No giant cells were seen on the surfaces of the synovia, nor were any multinucleated foreign-body giant-cell reactions present. The presence or amount of fibrinaceous stroma did not seem to correlate with the duration of arthritis.

Histochemical Stains

Stains for fibrin, collagen, reticulin, and elastin were done on the 8 synovia with acidophilic material. As demonstrated by Putt's stain, fibrin, which was confined mainly to villi, was found to be the major component of this material. With trichrome stains, these areas were fuchsinophilic (a rich magenta), which contrasted with the green staining of collagen. Sclerosis of villi was not seen, and reticulin was absent in these areas of the synovia. The fibrinaceous material was PAS-positive, and stains for amyloid were uniformly negative. The obliterative microvascular lesions were negative for fibrin by Putt's stain. Large numbers of mast cells were seen primarily in areas with few lymphocytes and plasma cells. They were particularly prominent in areas of angiogenesis.

In an effort to find spirochetes, Dieterle silver impregnation stains were done on all Lyme disease synovia. In 2 cases, spirochetes were seen within edematous vascular beds and in one vessel wall (Figures 5 and 6); they were not found in the fibrinaceous stroma. Spirochetes were not present in large numbers, and a meticulous search of many oil immersion fields was required to demonstrate them. They were seen only in synovectomy specimens and were not demonstrated in the small closed biopsy samples.

The Selection of Lyme Synovia From an Unknown Group

The H&E sections of the 17 Lyme disease specimens and 55 synovectomy specimens from other synovial diseases were coded and read blindly by two pathologists. Of the 72 cases, observer Y.J. diagnosed 14 as likely to be Lyme disease (Table 1) because of the presence of broad fields of fibrin matrix in as many as 50% of the synovial villi. P.D. thought that Lyme disease was present in 13 cases for the same reason. On the basis of this finding, both observers correctly identified 5 of the 8 Lyme synovectomy specimens, but incorrectly included several cases of rheumatoid arthritis, Reiter's dis-

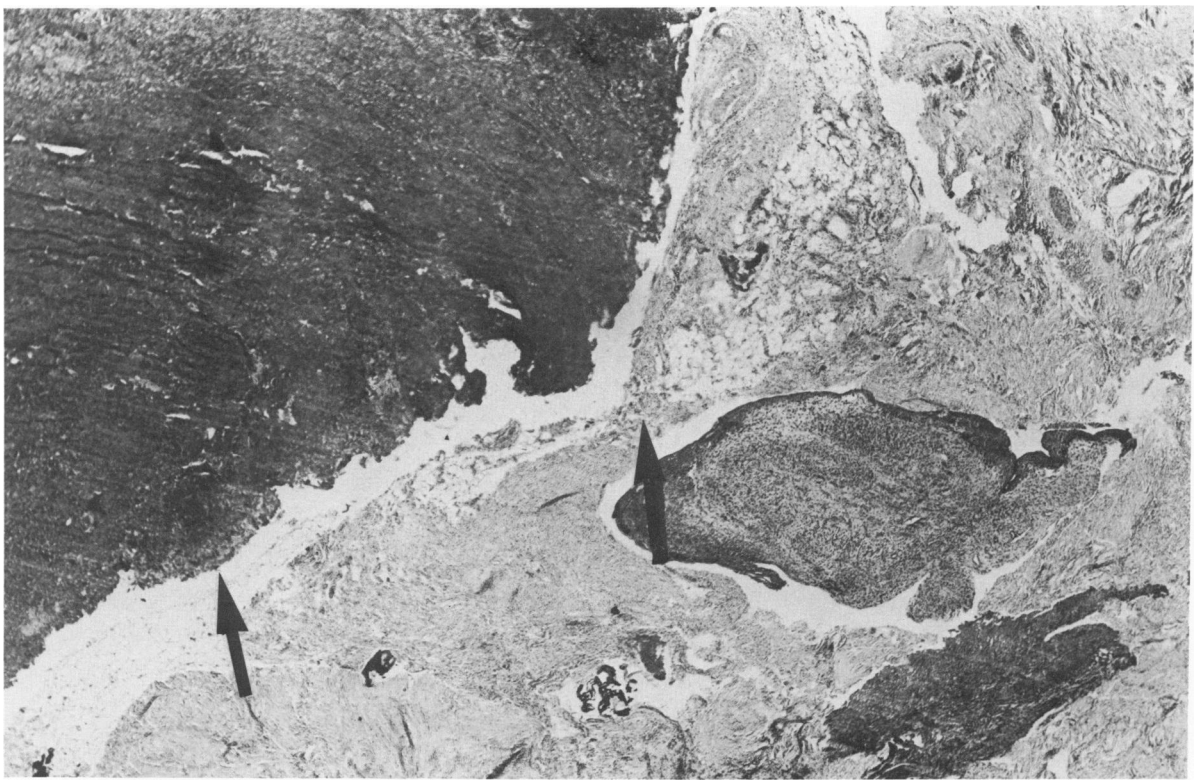

Figure 4—Mallory trichrome staining showing the darkly staining fuchsinophilic fibrinaceous stroma which may constitute up to 50% of the volume of surgical synovectomy specimens in Lyme arthritis. (×20)

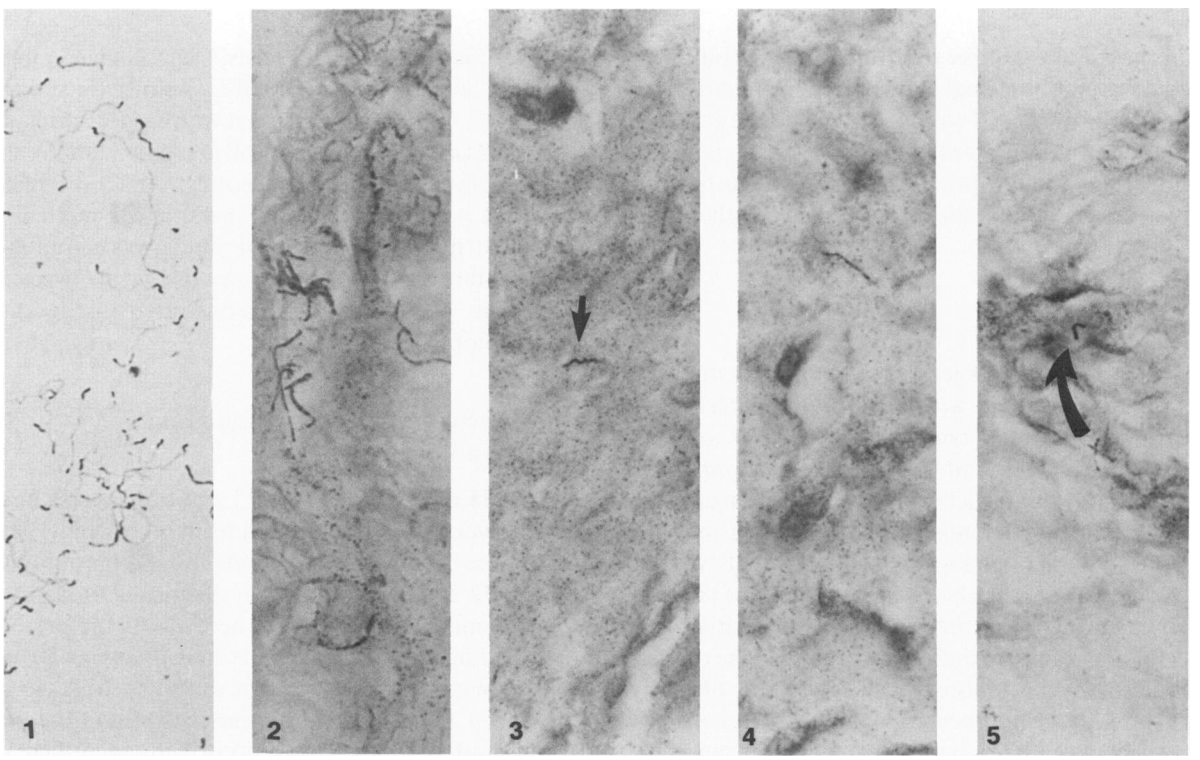

Figure 5—The Lyme disease spirochete is *Borrelia*; its length may be 39 μ. Contrast the morphology of Dieterle-silver-stained spirochetes in synovium, seen in panels 2–5, with cultured spirochetes maintained in Kelley's medium, seen at the far left. (×900)

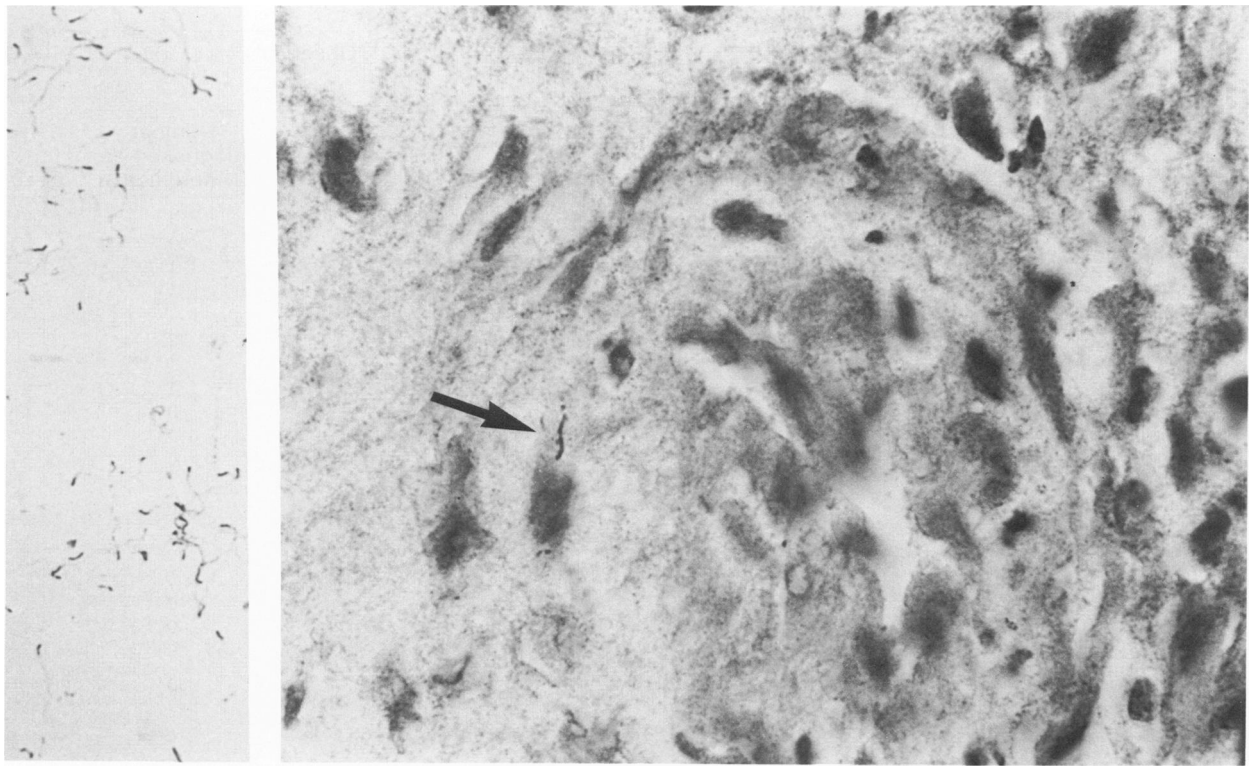

Figure 6—The Lyme spirochete tends to be located near vessels, and is seen in the wall of this thickened vessel. Maintenance control culture is seen at the left. (Dieterle silver impregnation stain, ×900)

ease, and chronic synovitis of unknown type. The remaining 9 Lyme synovia obtained by needle biopsy were not thought to be due to Lyme disease, because these specimens showed only chronic inflammation. Thus, disregarding vessel change, the majority of Lyme synovitis was separated from other synovial diseases by the presence of extensive fibrin deposits. The few cases of rheumatoid arthritis and Reiter's disease caused confusion because of fibrin pseudomembrane on synovial surfaces.

Cases of classic rheumatoid arthritis, trauma, giant-cell villonodular synovitis, and suppurative arthritis, were readily identified. Patients with rheumatoid arthritis had either palisading necrobiotic granulomas in the synovia, or had hyperplastic villi with lymphoplasmacellular infiltration. Minor differences were seen between the rheumatoid necrobiotic granuloma centers and the Lyme synovial fibrinaceous stroma (Table 2). Unlike rheumatoid necrobiosis, the LD fibrinaceous matrix material contained intact fibroblasts and other mononuclear cells, which were not palisading. The necrosis in RA appeared frankly necrotic and granular, whereas the acidophilic material in LD was less granular and more fibrillar. Coarse reticulin fibers were abundant within rheumatoid necrobiotic granulomas, whereas reticulin fibers were absent in Lyme fibrinaceous stroma (Figure 7). Nonspecific vascular wall thickening could be found in RA, but was not the concentric obliterative fibroplasia of LD. RA synovia lacked hemosiderin deposition, which was seen in 7 of the 8 surgically excised LD specimens and in traumatic arthritis synovia. Spirochetes were seen in 2 LD synovia, but in none of the other synovial diseases.

Discussion

The 17 patients in this study had severe Lyme disease, and most of them had chronic arthritis in knees at the time tissue was obtained. As noted in the past, the synovium in these patients had histologic similarities to rheumatoid synovium.[2,6,7] In both diseases, the synovium showed villus hypertrophy, vascular proliferation, and a moderate to marked infiltrate of lymphocytes and plasma cells, sometimes with lymphoid follicles. In the current study, we found vascular changes and fibrinaceous villus–stromal deposition that had not been noted previously. The acidophilic villus material was composed largely of fibrin (and possibly fibrinogen). However, it is not clear whether fibrin or fibrinogen, which is increased in exudative fluids, was deposited

Table 1—Selecting Lyme Synovitis From Other Synovial Diseases in a Blinded Study

Actual diagnosis	Number examined	Observer Y.J. Number diagnosed as Lyme disease	Y.J. %	Observer P.D. Number diagnosed as Lyme disease	P.D. %
Lyme disease (synovectomy)	8	5	62	5	62
Lyme disease (needle biopsy)	9	0	0	0	0
Reiter's disease	2	1	50	1	50
Rheumatoid arthritis	12	4	33	3	25
Chronic synovitis of unknown type	10	4	40	2	20
Trauma	4	0	0	1	25
Hemarthrosis due to hemophilia	1	0	0	1	100
Acute suppurative arthritis	5	0	0	0	0
Psoriatic arthritis	2	0	0	0	0
Pseudogout	1	0	0	0	0
Pigmented villonodular synovitis (giant-cell synovitis)	7	0	0	0	0
Xanthogranulomatous tenosynovitis	1	0	0	0	0
Degenerative joint disease (osteoarthritis)	6	0	0	0	0
Tendon sheath fibroma	1	0	0	0	0
No histologic abnormality	3	0	0	0	0
Total	72	14	19	13	19

on and within the villi as a first step in the damage process, or whether these proteins were deposited on collagen fibers that had been previously damaged by the action of collagenase.[7] The deposition appeared not to be exudative, because no more than a few inflammatory cells were observed in the fibrin matrix.

The inciting event for the stromal fibrin deposition is not clear. If it were due to a single, acute hemorrhagic episode, healing by scar tissue would be expected. However, collagen sclerosis was not observed in the synovial villi, nor was there histologic evidence for multinucleated giant cell reactions in any section. Therefore, we suspect that small episodes of microvascular bleeding occur intermittently as a consequence of small vessel damage, with repeated cycles of fibrin deposition. Although fibrinaceous villus stromal deposition was often abundant in Lyme disease, this finding seems to be nonspecific. As shown in the blinded study, it may also be found in Reiter's disease, rheumatoid arthritis, and in hemarthrosis due to hemophilia.

Microvascular proliferation is also a nonspecific and ubiquitous finding in inflamed synovial tissue. In this study, in both rheumatoid and Lyme synovium, a wide spectrum of angiogenesis was observed, ranging from budding capillaries in subsynovial granulation tissue to prominent, red cell-congested congeries of dilated vessels reminiscent of true capillary hemangiomas. Numerous mast cells were observed in these rich capillary beds, a finding noted previously in other conditions.[18] In addition, vascular thickening due to hypertrophy of small vessel and capillary walls was found. This response may be seen in any tissue bed damaged by inflammation, ulceration, or fibrosis.

In 5 cases of Lyme disease, we observed a specific microvascular lesion that was not found in the other synovial diseases. This lesion, a form of endarteritis obliterans, resembled the hypertrophic renal arteriolosclerosis of malignant hypertension, and the concentric adventitial fibrosis of small vessels in the spleen in systemic lupus erythematosus.[19] Obliterative endarteritis may also be seen in all stages of syphilis.[20] In Lyme disease, this lesion seems not to result from a direct immune attack upon the vessel. Although sparse lymphoid cells were seen in the adventitial regions of the involved vessels, no active endothelial cell damage or fibrin thrombi were seen.

The most important finding in this study was the

Table 2—Histochemical Reactions of Fibrinaceous LD Strome Versus RA Necrobiotic Granuloma

Stains for	LD	RA
Fibrin	+ + +, replacing villi	+, surface and spotty in nodules
Collagen	0 in villi	+ + in some villi
Fuchsinophilia	+ + +	+ +
Elastin	±	±
Reticulin	± to 0	+ + +, coarse fibers

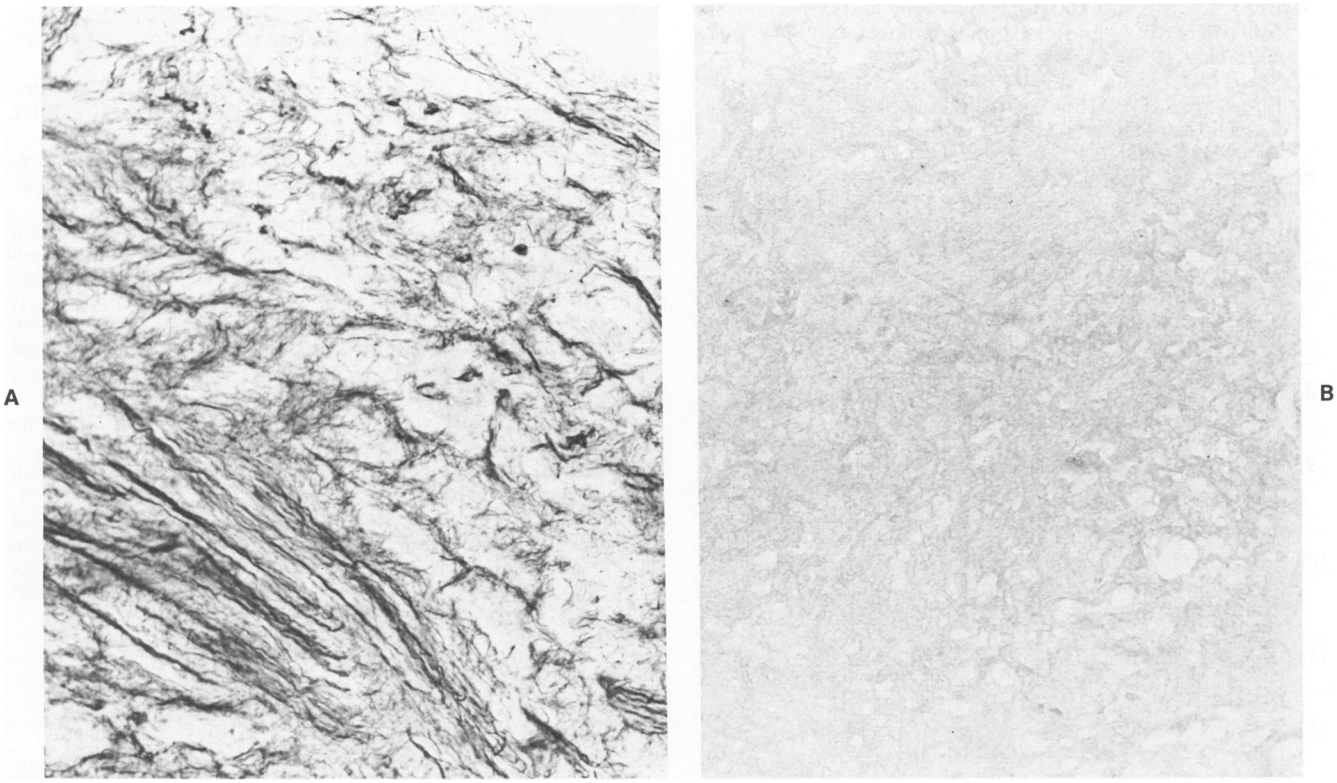

Figure 7—Coarse reticulin fibers were demonstrated in necrobiotic granulomas of rheumatoid arthritis (**A**). Reticulin fibers were not seen in Lyme disease fibrinaceous stroma (**B**). (Foote's reticulin stain, ×40)

demonstration of spirochetes both within the walls of thickened vessels and in the loose tissue in vascular beds in 2 of 8 synovectomy specimens. The Lyme disease spirochete, which is an organism of the genus *Borrelia*,[21,22] is longer (up to 39 μ) and less tightly coiled than *Treponema pallidum*, the agent of syphilis. Because of its length, one rarely sees an entire spirochete in affected tissue; more commonly, only portions of an individual organism may be visible in tissue. Even so, we suspect that the number of organisms in affected synovia is very small. It was necessary to examine many microscopic fields to find a few.

The Lyme spirochete has been cultured from blood and skin specimens of erythema migrans early in the illness.[9] We suspect that the spirochete may spread hematogenously to many different sites at that time; it has been recovered from cerebrospinal fluid two and a half months after the onset of the disease.[9] However, the organism has not yet been cultured from synovium; and therefore it has been unclear whether Lyme arthritis results directly from the presence of live spirochetes in synovium or whether it is an indirect immune response triggered by previous spirochetal infection. This study provides a major clue to the answer to this question, because for the first time, spirochetes were seen within edematous vascular beds and one vessel wall from synovial samples of 2 patients. This finding implies that that the Lyme spirochete may survive for years in affected synovium. This observation is in agreement with the recent finding that high-dose intravenous penicillin is effective for the treatment of established Lyme arthritis.[23] Furthermore, it seems likely that the spirochete may be directly responsible for the microvascular injury, the most characteristic feature of Lyme synovia.

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Acknowledgments

We appreciate the technical laboratory assistance of Adele Kusnitz, John Ryan, and Robert L. Grodzicki; the manuscript typing by Maria Warner; and the photomicrographs by Robert Specht. We also acknowledge the cooperation of Carl J. O'Hara, MD, of the New England Deaconess Hospital for allowing us to review pathologic material of one patient.