MICROBIOLOGICAL REVIEWS, Dec. 1986, p. 381–400
0146-0749/86/040381-20$02.00/0

Vol. 50, No. 4

Biology of *Borrelia* Species

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INTRODUCTION

Relapsing fever was known to the physicians of ancient Greece and possibly to those of an earlier civilization in Egypt (92, 180). Extensive epidemics of louse-borne relapsing fever occurred in the ensuing centuries, including the 20th (55). Like plague and cholera, the disease has affected human societies and events. Lenin is said to have remarked, in reference to the outbreaks of louse-borne typhus and relapsing fever rampant during the Russian Revolution, that "either socialism will defeat the louse or the louse will defeat socialism" (224).

In the first half of this century, relapsing fever was of considerable interest to microbiologists, not only because of the continuing morbidity and mortality it was causing, but also because of the recognition by several early immunologists that the antigenic variation of relapsing fever was a useful model for studying the immune system. During World War II and its aftermath there were again the conditions of famine and displaced populations that spawn epidemics of relapsing fever. However, this period also saw the mass production of penicillin and DDT. These technological advances provided both an effective treatment and an effective control measure for the disease. Although louse-borne relapsing fever may be on the upsurge again in famine-struck areas of Africa, it has been comparatively quiescent in the world now for almost 40 years. As a result, the number of scientific articles on relapsing fever and borreliae plunged from 50 or more a year before 1950 to less than 10 a year on average since then. Another consequence of this change in attention has been a great reduction in the number of "borreliologists" in the world. Investigators such as Koch, Leishman, Noguchi, Ehrlich, and Metchnikoff at one time were among that number.

It is ironic then that, following the decline in study of this group of bacteria, at least one (and probably two) important diseases should be recognized as being caused by members of the species *Borrelia*. In addition, it appears that those early immunologists were insightful: the antigenic variation evidenced by the relapsing fever borreliae is of basic biological interest. Because of this renewal of curiosity about borreliae, we have attempted to provide a primer on the biology of this genus and, to some extent, acquaint investigators of this generation with the large body of works of past generations.

Felsenfeld's review in this journal in 1965 (91) and his book in 1971 (92) thoroughly covered details on the epidemiology, epizootology, and entomology of relapsing fever *Borrelia* spp., their hosts, and vectors. We focus in these pages on the organisms themselves and on their taxonomy, ultrastructure, physiology, cultivation, and biology in arthropod and vertebrate hosts. The presentation of vector and host biology does not detail every aspect of every species. Instead, we restrict our descriptions to some host-vector-parasite interactions that are particularly well characterized. Although the exemplifying biological systems come, for the most part, from the group of relapsing fever borreliae, we think that the lessons learned from this group of organisms provide a knowledge base for present and future studies of the two most recently discovered *Borrelia* species: the Lyme disease agent (61) and the probable cause of epizootic bovine abortion (171, 196, 220).

DESCRIPTION OF THE GENUS AND TAXONOMIC CONSIDERATIONS

Borreliae are spirochetes and as such have in common with other spirochetes the following structural characteristics (129, 142). (i) The cells are helically shaped and motile with three modes of movement. (ii) An outer cell membrane surrounds the protoplasmic cylinder complex, consisting of the cytoplasm, the inner cell membrane, and the peptidoglycan. (iii) Flagella, which are equivalent to other bacterial flagella in architecture, are located not at the cell's surface but in the periplasmic space between the outer cell membrane and the protoplasmic cylinder. These periplasmic flagella are inserted at the termini of the protoplasmic cylinder.

Spirochetes have traditionally been distinguished from other eubacteria at the taxonomic level of order (*Spirochaetales* [165]). Nevertheless, the heterogeneity of spirochetes in their physiologies and their guanosine-cytosine contents (27 to 66%) suggests that their phylogenetic origin was at an even deeper level. Ribosomal ribonucleic acid (RNA) cataloging has, in fact, shown that spirochetes represent an ancient grouping and that a formal rank of class or division (phylum) would be more appropriate than order for this unique collection of microorganisms (96, 198).

Ribosomal RNA cataloging and oligonucleotide mapping

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TABLE 1. *Borrelia* species associated with diseases of humans and domestic animals

Borrelia sp.[a]	Disease	% DNA homology with *B. hermsii*[b]	Arthropod vector[c]	Geographic distribution of disease
B. hermsii	New World tick-borne relapsing fever	100	*O. hermsi*	Western United States and Canada
B. turicatae	New World tick-borne relapsing fever	86	*O. turicata*	Southwestern United States and northern Mexico
B. parkeri	New World tick-borne relapsing fever	77	*O. parkeri*	Western United States and Baja California
B. mazzottii	New World tick-borne relapsing fever	ND	*O. talaje*	Mexico and Central America
B. venezuelensis	New World tick-borne relapsing fever	ND	*O. rudis*	Central America and northern South America
B. duttonii	Old World tick-borne relapsing fever	17	*O. moubata*	Sub-Saharan Africa
B. crocidurae	Old World tick-borne relapsing fever	32–35	*O. erraticus*	North Africa, East Africa, Near East, Middle East, and southeast Europe
B. persica	Old World tick-borne relapsing fever	ND	*O. tholozani*	Middle East, Greece, and Central Asia
B. hispanica	Old World tick-borne relapsing fever	ND	*O. marocanus*	Iberian peninsula and western North Africa
B. latyschewii	Old World tick-borne relapsing fever	ND	*O. tartakowskyi*	Iran, Afghanistan, south-central USSR, Iraq, and southwestern USSR
B. caucasia	Old World tick-borne relapsing fever	ND	*O. asperus*	Iraq and southwestern USSR
B. recurrentis	Louse-borne relapsing fever	ND	*Pediculus humanus*	Worldwide
B. anserina	Avian borreliosis	53–63	*Argas persicus* and other *Argas* spp.	Worldwide
B. theileri	Bovine borreliosis	ND	*Boophilus microplus* *Boophilus annulatus* *Boophilus decoloratus* *Rhipicephalus evertsi*	South Africa, Nigeria, Australia, Brazil, and Mexico
B. burgdorferi	Lyme disease	30–44	*I. dammini* *I. ricinus* *I. pacificus*	Eastern United States Europe, western USSR, and Scandinavia Western United States
B. coriaceae	Epizootic bovine abortion (?)	44–50	*O. coriaceus*	California

[a] References 60, 143, 155, and Johnson et al. (in press).
[b] References 138, 143, 144, 218, Hyde (Ph.D. thesis), and Johnson et al. (in press). ND, Not determined.
[c] References 58, 60, 130, 171, 220, and 226.

of several spirochetal genera have confirmed the impression that they are diverse as a group (198). As the leptospiral group's distinctive structure and metabolism seemed to indicate, a completely separate placement (family) for leptospires was justified. Within the large group of remaining spirochetes (family *Spirochaetaceae*) are the borreliae. They share with other family members the use of ornithine as the diamino acid in the cross-linking peptide of peptidoglycan (162). Other representatives of this family are the treponemes, which also have their pathogenic varieties. However, on the basis of ribosomal RNA relatedness and deoxyribonucleic acid (DNA) homology, there is no reason to consider borreliae more akin to the host-associated treponemes than they are to free-living spirochetes in the genus *Spirochaeta* (198).

Ecological and biochemical characteristics that serve to identify the genus *Borrelia* are these. (i) All species in this genus are transmitted to vertebrates by hematophagous arthropods; there often is transovarial transmission of the borreliae in arthropods. (ii) The guanosine-cytosine content of the genomic DNA is between 27 and 32% (138, 143, 144, 217; F. W. Hyde, Ph.D. thesis, University of Minnesota, Minneapolis, 1985; R. C. Johnson, W. Burgdorfer, R. S. Lane, A. G. Barbour, S. F. Hayes, and F. W. Hyde, Int. J. Syst. Bacteriol., in press).

Table 1 lists the majority of known *Borrelia* species. Some named species which have not been very well characterized or which lack a clear association with human or domestic animal disease were not included. These other species (and their associated *Ornithodoros* sp. tick vectors) are *Borrelia graingeri* (*Ornithodoros graingeri*) and *B. tillae* (*O. zumpti*) of southern Africa, *B. brasiliensis* (*O. brasiliensis*) of Brazil, and *B. queenslandica* (*O. gurneyi*) of Australia. The list was compiled from catalogs of Kelly (155), Burgdorfer (60), and Hoogstraal (130).

Borreliae have often been classified according to the specificity of the parasite-vector relationship. For instance, the species *B. duttonii* and *B. anserina* cannot be efficiently transmitted by each other's natural vectors, *O. moubata* and *Argas persicus*, respectively (92, 190). Three species of borreliae found in western and southwestern North America, *B. hermsii*, *B. turicatae*, and *B. parkeri*, also have shown complete specificity for their arthropod vectors, *O. hermsi*, *O. turicata*, and *O. parkeri* (51, 80–82, 157); *B. hermsii* can be transmitted by *O. hermsii* but not by *O. turicata* or *O. parkeri*, even if all three species were feeding on the same spirochetemic host. Nonetheless, because these three types of *Borrelia* share at least 77% DNA homology with one another (143; Hyde, Ph.D. thesis), there is some justification for putting them in a single species.

The DNA hybridization approach to determining relatedness between these species has just begun. The DNA homologies will likely be considered in concert with biological features, such as vector specificity, metabolic characteristics, such as sugar fermentation (225), and structural traits in any revamping of the taxonomy.

Another phenotype that was judged by previous investigators to be important for any classification scheme for borreliae was the range of mammals that were susceptible to the pathogens (18, 19, 50). Although it was realized that strains within a species could vary in virulence (106) and that one could not be absolutely definite about whether a given animal was infected or not, a consensus was obtained for many types of borreliae as to what experimental animals they were each capable of infecting (18, 74). Some of these relationships are worth relating, for they may, in addition to their purely taxonomic functions, offer clues that lead to the identification of virulence factors. One such often-noted finding was the impossibility of infecting guinea pigs (18, 83), even the newborn, with *B. duttonii*, a species that readily produces heavy infections in mice (108). In contrast, the species *B. hispanica* and *B. persica* do reproducibly cause massive infections in guinea pigs (2, 12, 14, 52, 70, 84, 208). *B. recurrentis*, the agent of louse-borne relapsing fever, is another special case. Man seems to be the only mammalian host for this organism in nature (20, 74, 161, 248). Primates can be readily infected with this group of organisms and experience relapses (20, 161). It has been difficult, however, to find any other animal which will show relapses during the illness. Newborn or splenectomized animals have the most clear-cut infections, but the spirochetemias are usually only of a short duration (17, 20, 161, 228). *B. anserina* seems capable of producing significant infections only in birds (234). As is true of other *Borrelia* spp., infections with *B. anserina* are more severe in very young animals than in adults (169).

Another criterion that has been used for classifying borreliae is their ability to infect and to be transmitted by arthropods which are not their normal vectors. For instance, *B. duttonii*, *B. crocidurae*, *B. turicatae*, and *B. parkeri* multiplied in lice, but *B. hispanica*, *B. persica*, and *B. latychevi* could not (125).

Cross-immunity has also been used for taxonomic purposes (156, 159, 188, 216). However, because of the antigenic variation that many species manifest and the lack of standardization of either immunization protocols or challenge procedures, these data may not be reliable enough for classification of species.

As we study the most recently discovered species, *B. burgdorferi* and *B. coraceae*, characteristic host ranges are also becoming apparent. Evidence of infection by *B. burgdorferi*, for instance, has been found in a variety of feral mammals and birds (6, 7, 47, 48, 177, 178; J. F. Anderson, R. C. Johnson, L. A. Magnarelli, and F. W. Hyde, Zentralbl. Bakteriol. Mikrobiol. Hyg., in press; E. M. Bosler and T. L. Schulze, Zentralbl. Bakteriol. Mikrobiol. Hyg., in press; R. S. Lane and W. Burgdorfer, Zentralbl. Bakteriol. Microbiol. Hyg. 1 Abt. Orig. A, in press). The very plethora of potential reservoirs has hindered identification of the critical reservoir for the disease in nature.

STRUCTURE

Earlier investigators of borreliae and other spirochetes used light microscopy to study the structure of these bacteria. Most data on spirochetal structure date from the microscopic descriptions by Zueler, Dobel, and Noguchi (reviewed in reference 129) and later by DeLamater et al. (85, 86), Pillot et al. (203, 204), and Rose and Morton (211). Beginning in the 1940s, workers in this field applied electron microscopy to unravel spirochetal architecture (15, 114, 115, 152, 174, 238). The contributions of Hovind-Hougen over more than a decade have revealed many features of borrelial structure that were hitherto unknown (133–136, 150). Biochemical and immunochemical approaches to structure elucidation have also brought a further understanding of the borrelia's surface and of some subcellular organelles.

Borrelia spp. vary in length, diameter, tightness of the coils, regularity of the coils, and number of periplasmic flagella. The length can range from 8 μm in the case of *B. coriaceae* sp. nov. (171; Johnson et al., submitted) to more than 20 to 30 μm in *B. burgdorferi* (61, 136). The widths of the helices range from that of *B. burgdorferi*, which is the narrowest at 0.2 to 0.3 μm (61, 136), to those of *B. recurrentis* and *B. persica*, which are reported to have diameters of 0.45 to 0.5 μm (133, 150). As a rule, the width measurements of a particular *Borrelia* sp. tend to be more reproducible from one determination to another than those of length (78). Cell length is a function of the age of the cultures; the lengths become longer as cells reach the stationary phase of growth (9). Length can also vary with the nutritional adequateness of the culture medium or experimental animal in which the borrelia finds itself. Addition of glucose to spirochetes suspended in an energy-depleted medium is enough to reduce the length of the cells as the amplitude of the coils increases (A. G. Barbour, unpublished observations).

Approaching the structure of the borrelia from the outside, we encounter first the cell's surface. Borreliae do not have a discernible regular array at the surface (129, 133–136, 150), but may have an amorphous slime layer at this position (133–136, 238). Whether this layer represents incorporated host or medium components or, alternatively, substances produced by the cell itself remains to be determined. In any case, such a layer seems to be only weakly attached to the *Borrelia* spp. that have been examined so far. The amorphous layers shown in Fig. 1 are lost upon washing with phosphate-buffered saline (S. F. Hayes, unpublished observations). The reactivity of this layer with Alcian Blue indicates that carbohydrates are present in this layer (232).

On the journey inwards, the next met structure, and one assuredly part of the borrelial cell, is the outer cell membrane or outer sheath. This membrane has the trilaminar organization common to ctyoplasmic membranes (4, 129, 134, 135, 142, 150). The fluidity of this membrane, and its dissimilarity to the outer membrane of gram-negative bacteria (118), is attested to by two observations. One was the finding that the outer membrane of *B. hermsii* and *B. burgdorferi* was separated from the underlying protoplasmic cylinder and then solubilized by very dilute solutions of sodium dodecyl sulfate (162; J. C. Coleman, J. L. Benach, G. Beck, and G. S. Habicht, Zentralbl. Bakteriol. Mikrobiol. Hyg., in press) or by nonionic detergents (35); the membranes were reconstituted by dialysis against water (162). The second indication of a comparatively fluid outer membrane was the phenomenon of cell "patching" or "capping" (33). Capping was seen when an antimembrane protein antibody together with a second ligand were bound to cells (Fig. 2). When the cells were fixed with formaldehyde before exposure to antibodies, the two-dimensional aggregation of proteins was not seen. Charon and co-workers, who used

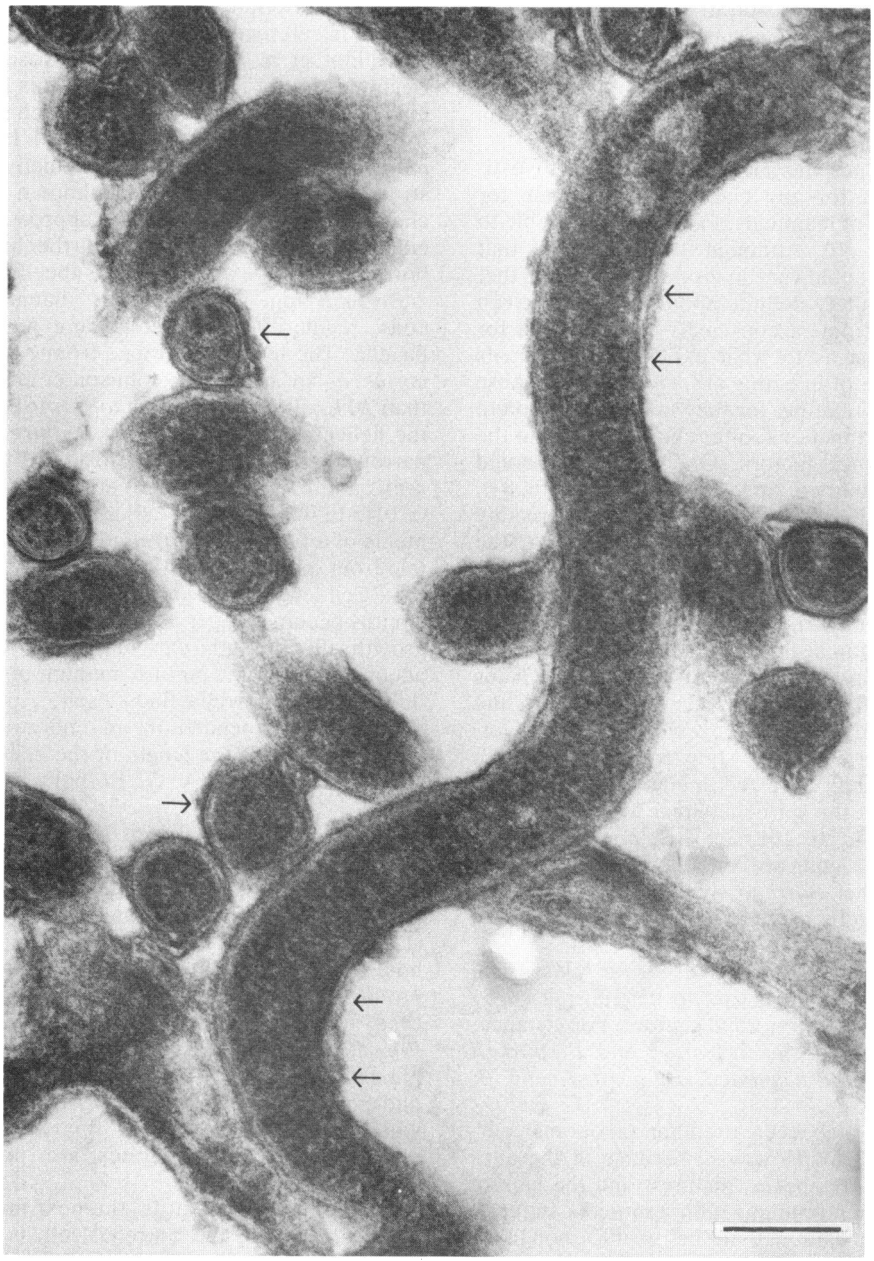

FIG. 1. Electron photomicrograph of a thin section of *B. burgdorferi* cells stained with Alcian Blue. Arrows indicate the patchy distribution of amorphous material associated with the outer membrane of the spirochete. Bar, 0.2 μm.

another approach, had previously demonstrated the fluid nature of a leptospire's outer sheath (68).

Lipopolysaccharide or the spirochetal equivalent to endotoxin has been sought by several investigators. These studies were often prompted by a desire to understand the pathogenesis of the Jarisch-Herxheimer reaction, which in clinical practice is often seen soon after antibiotics are given to patients with relapsing fever (8, 55). The shocklike state that sometimes ensues reminded several investigators of the physiologic reactions induced by administration of endotoxin. In spite of this resemblance, many attempts to find a significant amount of endotoxin activities in borreliae and pathogenic treponemes produced little direct evidence (8, 65, 104, 119, 218, 249). More recently, however, a lipopolysaccharide with endotoxinlike properties has been described in *B. burgdorferi* (35, 100). Preliminary studies indicated that the *B. burgdorferi* lipopolysaccharide that was extracted was of the "rough" variety (35).

Isolated outer membranes from *B. hermsii* and *B. burgdorferi* contain 45 to 62% protein, 23 to 50% lipid, and 3 to 4% carbohydrate (162; Coleman et al., in press). Major contributors to the protein fractions in the outer membranes of these two species have been identified. *B. hermsii* has a single, abundant, surface-exposed protein associated with the outer envelope (24, 30, 32). The generic name for this protein is variable major protein (VMP). Each serotype of a given strain of *B. hermsii* has a VMP that is characteristic in its size, primary amino acid sequence, and reactivity with antibodies (25, 32, 35). The VMP, therefore, confers serotype specificity to a cell; the role of the VMPs of

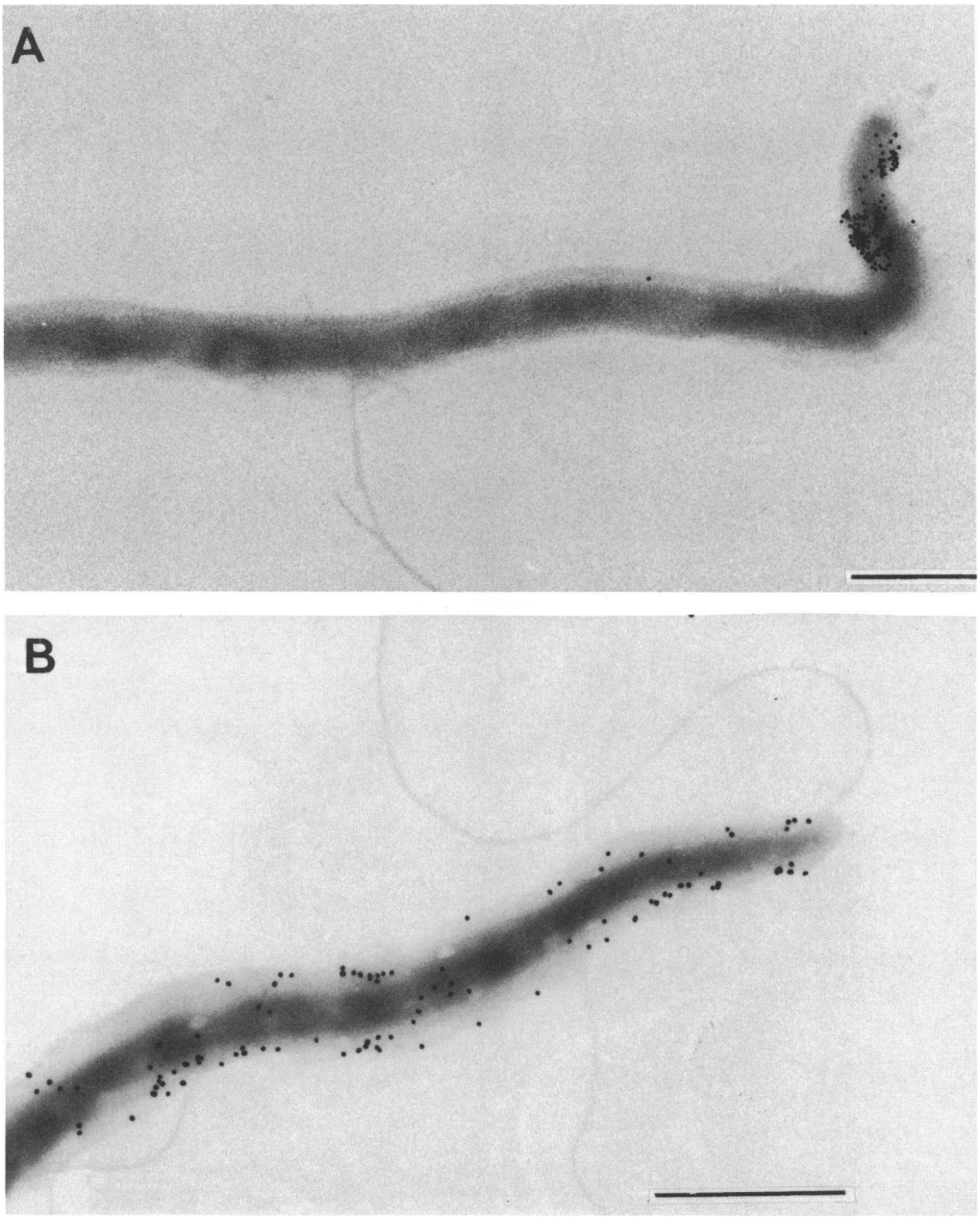

FIG. 2. Labeling of *B. burgdorferi* B31 with a monoclonal antibody (H5332) directed against the OspA outer membrane protein of this strain (33). Bound antibody was detected with a second ligand, protein A-coated colloidal gold. Labeling was performed under two different experimental conditions. (A) A spirochete that was fixed with formaldehyde after the reactions with antibody and second ligand; the protein A-colloidal gold complexes have aggregated at the end of the cell. (B) A spirochete that was fixed before exposure to antibody and the second ligand; in this case, the gold particles are more evenly dispersed over the surface of the cell. Bar, 0.5 μm. (Figure adapted from reference 33.)

relapsing fever borreliae in antigenic variation is discussed below. *B. burgdorferi* strains from North America usually have two major surface proteins, the OspA and OspB proteins (28, 29, 31, 33, 137). Like the VMPs, in situ OspA and OspB proteins are cleaved from the membrane by proteases (24, 31). OspA and OspB proteins vary among *B. burgdorferi* strains in their apparent molecular weights and antigenicities (28, 31, 230, 247; B. Wilske, V. Preac-Mursic, G. Schierz, and K. V. Buson, Zentralbl. Bakteriol. Mikrobiol. Hyg., in press).

The loose association of the outer envelope with the underlying protoplasmic cylinder leads to the separation of these cell components when borreliae and treponemes are put in hypotonic solutions (Fig. 3) (241). Outer envelope blebs are also seen when specific antibody and a complement source are added to borreliae (156), when cells are frozen and thawed (175), when cells are exposed to penicillin (34), and in aged cultures (9). These findings indicate that disturbances to the cell can lead to large bleb formation. An alternative viewpoint was prevalent in the first half of this century. During this time many workers who viewed these large blebs, or "gemmae," thought that they represented another stage of a life cycle of the spirochetes (114, 115, 187); early classification of borreliae as protozoa encouraged

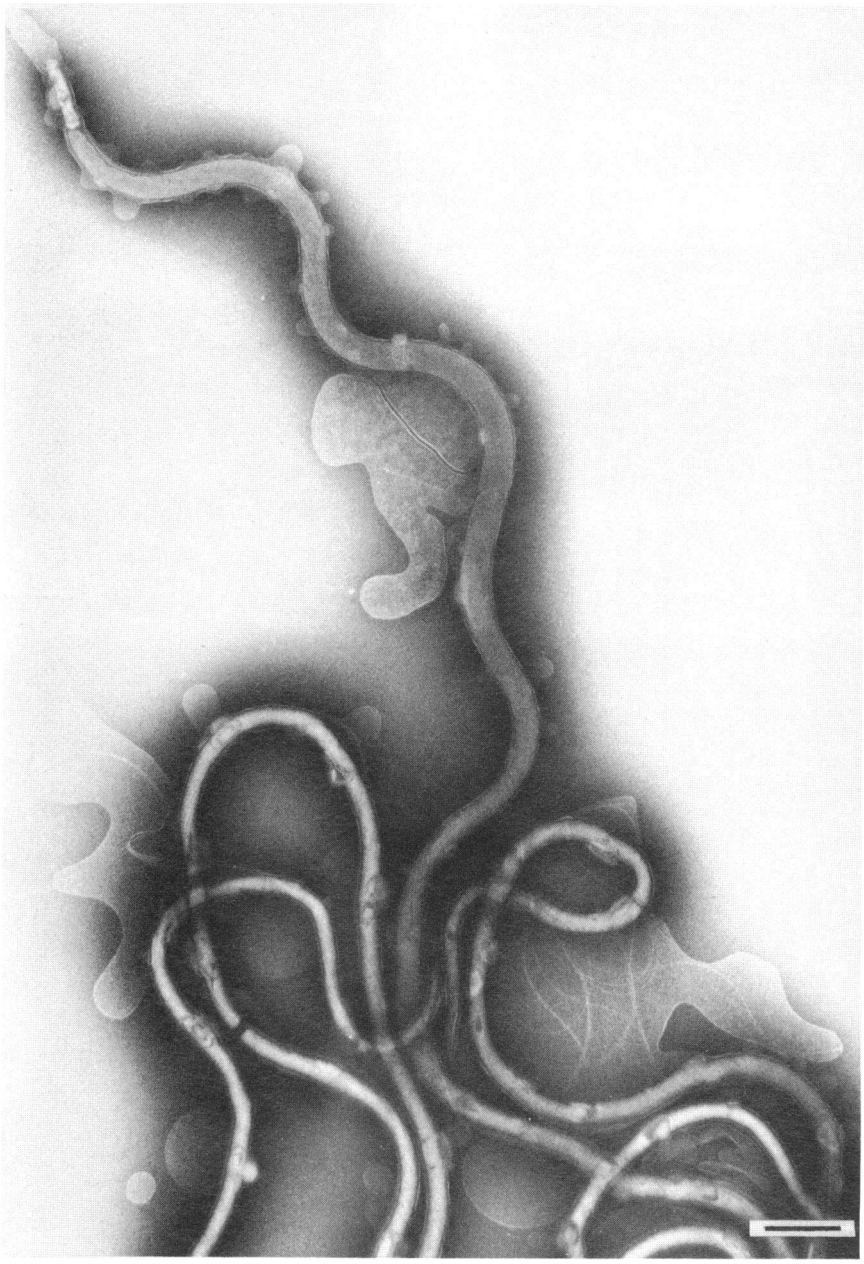

FIG. 3. Electron photomicrograph of *B. burgdorferi* cells which have been negatively stained with 3% ammonium molybdate, pH 6.5. Large and small blebs of the outer membrane are shown. In the large bleb in the right-hand corner released periplasmic flagella can be seen. Bar, 0.5 μm.

such hypotheses (193). The involuting mature spirochete was thought to produce small granules which were nonvegetative under adverse conditions but which in a more conducive environment would metamorphose into a full-length spirochete again (114, 115, 187). DeLamater et al. (85, 86) and Pillot et al. (203, 204) were careful students of changes during cultivation or infection in morphology of spirochetes and of the appearance of blebs or gemmae. Figure 4 shows a gemma containing numerous small structures ("granules") with trilaminar membranes surrounding them. The internal material of the granules appears to be similar in appearance and electron density to the nucleocytoplasm of the protoplasmic cylinder. The nature and function of such structures are unknown; they do not appear to be an artifact of block sectioning. But there has never been evidence that they harbor filterable, sporelike entities; when body fluids from infected arthropods and mammals were examined, only those fluids that contained intact, whole spirochetes were infectious to other animals (57, 124, 229, 246; B. J. W. Beunders, M.D. thesis, University of Leiden, Leiden, The Netherlands, 1932). Nevertheless, the ubiquitousness of these blebs and the granules prompts another critical examination of their natural history and the circumstances that bring about their formation.

Continuing our penetration of the borrelia, we come upon the periplasmic flagella beneath the outer membrane. There

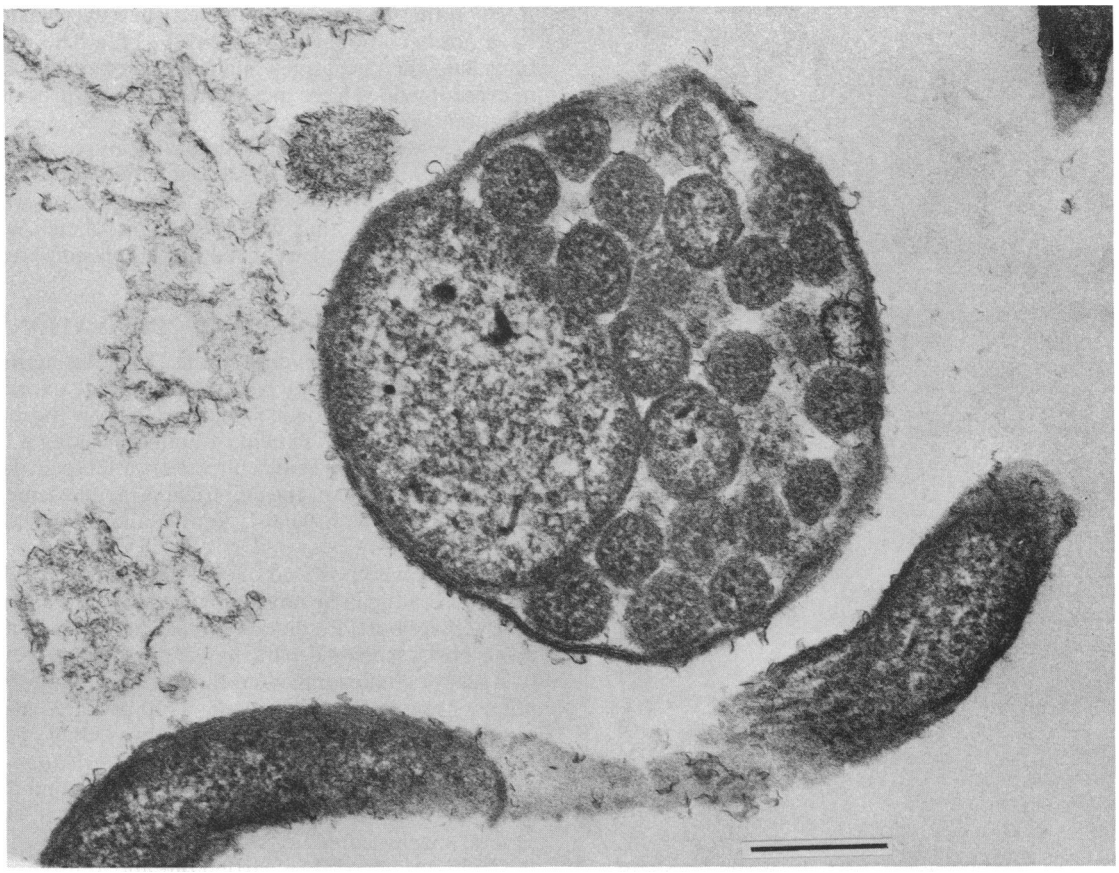

FIG. 4. Electron photomicrograph of a thin section of a round gemma containing the protoplasmic cylinder of the spirochete and several smaller granules. This structure was noted in an in vitro cultivated population of *B. burgdorferi*. Bar, 0.2 μm.

is every indication that these flagella mediate spirochete motility (39), but the actual mechanisms of borrelial movement will not be further considered. Borrelial flagella resemble one another and basically have the same architecture as flagella of other eubacteria (129). Like other spirochetes' flagella, borrelial flagella insert subterminally and bipolarly (Fig. 5). Holt reviewed the anatomy of spirochetes and the architecture of spirochetal flagella in detail (129).

The orientation of the flagella insertions with respect to the long axis of the cell may differ between species. Hovind-Hougen found this to be the case with treponemes (134, 135). In *B. burgdorferi*, the flagellar inserts are parallel with the long axis (61, 136); in *B. coracei* sp. nov. the flagellar inserts are staggered and in offset rows (171).

Borrelia spp. also vary in the number of flagella they may have (61, 134–136). The number of flagella seen in cross section depends upon the location in the cell from which that section was taken. Because of the overlap of flagella in the middle, a section from the center part of the cell may have twice as many flagella as a section obtained from near the terminus of a cell. Examination of the number of insertion points in negative stain preparations has also been done (Fig. 5).

The relapsing fever borreliae generally have between 15 and 30 flagellae (134–136); isolates of *B. burgdorferi* have had 7 to 11 (61, 136). A single strain of *B. burgdorferi* can vary in the number of flagella a cell has (136; S. F. Hayes and A. G. Barbour, unpublished observations). This suggests that there can be phenotypic variation in this characteristic. In 1929 Aristowsky and Hoeltzer noted that a borrelia strain long maintained in culture became more elongated and less tightly coiled (11); this possibly was a consequence of a change in either the number or function of the flagella of the cultured strain.

The flagella has four components: filament, hook, neck, and basal disk (129, 174). Borrelial flagella, like treponemal flagella, resemble flagella of gram-positive bacteria (129, 136). While treponeme flagella are sheathed, borrelial flagella are characteristically unsheathed (134–136). At the points of insertion, the hook portion tapers into the narrower filament. The hook has a honeycombed appearance when subjected to some negative stains (27, 134, 135). The hooks of *B. crocidurae* and *B. recurrentis* were 50 nm long and 15 nm wide; the flagella of these species tapered in their necks to 9 nm in width (133). The hook of *B. hermsii* was 50 nm in length and about 10 nm in width (27). The basal disks of *Borrelia* spp. examined to date have been approximately 35 nm in diameter (27, 133).

Borrelia spp. share an antigen that is associated with their periplasmic flagella (27). This epitope was identified with a monoclonal antibody and is part of the flagellin proteins, the main constituents of the filamentous sections of the flagellar apparatus. The apparent molecular weights of the flagellins of *B. hermsii* and *B. burgdorferi* are 39,000 and 41,000, respectively (27).

Other than the confirmation that borrelial cell walls contain muramic acid (111) and the identification of ornithine as the diamino acid in the peptidoglycan (162), few investiga-

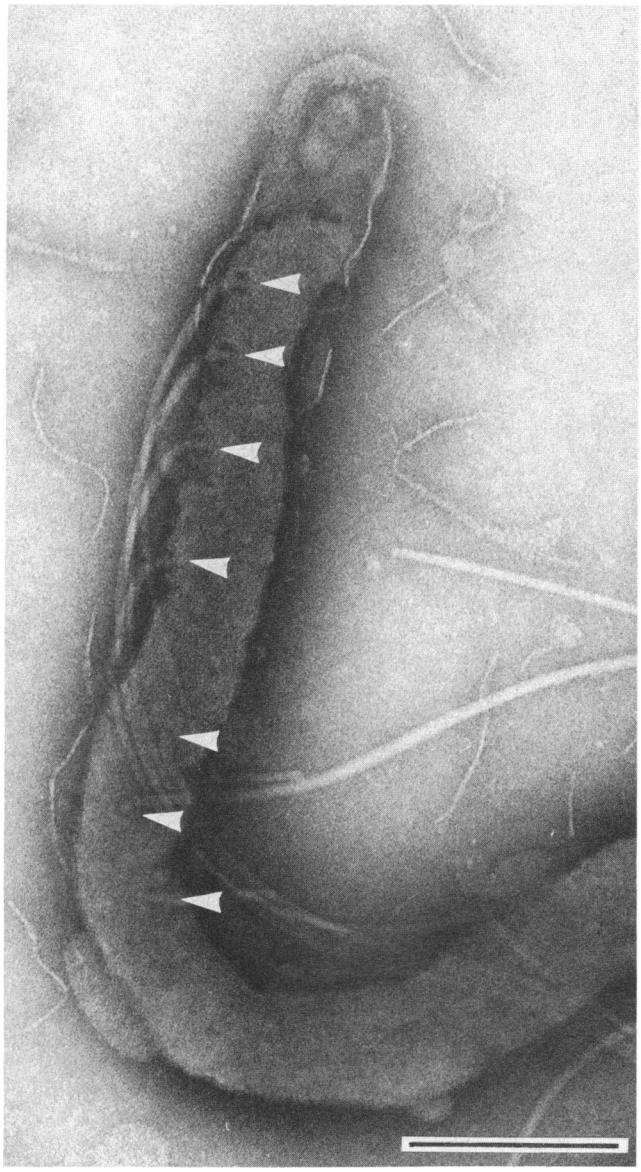

FIG. 5. Electron photomicrograph of the terminus of a negatively stained cell of *B. burgdorferi*. The seven periplasmic flagellar insertion points of this cell are indicated by arrowheads. The sample, after absorption to Parlodion film, (Mallinckrodt, Inc., St. Louis, Mo.) was treated with 1% sodium deoxycholate for 1 min, washed with water, and then stained with 3% ammonium molybdate. Bar, 0.2 μm.

tions have been made of the cell walls of *Borrelia* spp. This structure has been assumed to be the electron-dense layer just external to the cytoplasmic membrane of the protoplasmic cylinder (134, 135, 162). Further biochemical, immunochemical, and structural analyses of borrelial peptidoglycan are needed.

The handedness of the spirochete's helix is inherent in the protoplasmic cylinder. Pathogenic treponemes and borrelia species are consistently either left-handed or right-handed (134, 135, 234). Whether variants that have oppositely handed helices can arise from the parent strain is not known; there may be one example of such variation (123).

In treponemes unique microtubules are found within the cytoplasm (129, 134, 135, 142). In the several species of *Borrelia* that have been examined such cytoplasmic tubules have not been noted (129, 134–136, 142). The cytoplasm of borreliae when negatively stained and examined by electron microscopy does have mesosomelike structures of unknown function (4, 209, 238).

Our review of the ultrastructural features of members of the genus *Borrelia* leads us to conclude that the only reliable characteristics that serve to distinguish borrelia from treponemes, the other pathogenic spirochetes, are the absences from borreliae of both cytoplasmic tubules and flagellar sheaths.

METABOLISM AND CULTIVATION

Borrelia were discovered in the 1870s during the pioneering days of bacteriology (195). As would be expected, many attempts have been made since then to grow these organisms outside the diseased patient or animal. Taking a look at the successes and failures of cultivation attempts, we find that we can relate some of the empirical principles emerging from culture studies with what is known about borrelial metabolism.

As far as we know, no one has found a bonafide *Borrelia* sp. proliferating in an environment outside of a vertebrate or invertebrate host. Predictably, then, the nutritional needs for these host-associated parasites are complex. Nevertheless, two sets of studies indicated that growth requirements were not so stringent or host specific as to preclude successful in vitro cultivation. The first finding was that of Novy and Knapp who succeeded in growing *B. turicatae* in dialysis sacs in the peritoneum of rats (196). The second finding, and one made by several investigators, was that borreliae of several species could be grown in embryonated eggs and that this cultivation could be carried out apparently for unlimited passages (43, 67, 194, 207; G. E. Olisa, Ph.D. thesis, Bernhard-Nocht-Institut für Schiffs-und Tropenkrankheiten, Hamburg, Federal Republic of Germany, 1959). The other advantage for would-be cultivators is that borreliae seem to prefer an extracellular existence. Although there is some controversy about whether or not borreliae can thrive or persist inside cells, there is no evidence to suggest that they are obligate intracellular parasites.

A technique used by early cultivators was the placement of a paraffin or oil cap on the tubes of broth cultures (10, 139). Although this was first held as evidence that borreliae are anaerobic, further study showed that borreliae are microaerophilic (153, 170). The cap serves as much to prevent the loss of dissolved carbon dioxide in the medium as to limit incoming oxygen (10). In distinction to treponemes, which are anaerobic, borreliae are not sensitive to metronidazole (143). Borreliae contain an iron superoxide dismutase but not catalase or peroxidase (13).

Other investigators noted that borreliae became more motile in suspensions or grew better in media when glucose was added (53, 54, 248). Moreover, heavily spirochetemic animals were found to have plasma glucose concentrations 50% below and plasma lactate levels 150% above those values in uninfected animals (99). Studies using intact cells and analyses of cell extracts have shown that borreliae require glucose and ferment it by the Embden-Meyerhoff pathway; lactic acid is the predominant metabolic end product (99, 141). Some *Borrelia* spp. also seem capable of using fructose (99), maltose, trehalose, or raffinose (225). The salutary effect of sodium pyruvate on borrelia growth in vitro (153) seems to have as its corollary the significant stimulation to glycolysis it provides when present in low concentrations (99).

Successful medium formulations of the past also had in common the inclusion of serum, often rabbit, and either an albumin preparation or a fresh organ tissue extract (9, 69, 71, 103, 139, 161, 163, 170, 172, 179, 191, 248). We continue to use bovine albumin and rabbit serum in our medium (23, 26). The albumin preparation alone or in combination with rabbit serum provides the required long-chain fatty acids that are incorporated unaltered into the cellular lipids (143, 173, 202). A borrelia does not have the ability to elongate the chain or to beta-oxidize the fatty acids that are supplied (173). The fatty acid composition of borrelia cells reflects that which is present in the growth medium (173). Unsaturated, but not saturated, fatty acids supported growth of *B. hermsii* in medium with a fatty acid-free albumin preparation (154).

Cholesterol in the medium is also incorporated into cells (173, 202). In addition, an unusual cholesterol glucoside and its acylated derivative are synthesized from cholesterol by *B. hermsii* (173). Exogenous glucose is used for synthesis of these compounds as well as phospholipids (179).

Although several microbiologists in Europe, USSR, Israel, and North Africa were, with little doubt, successful in serially passaging in vitro a variety of *Borrelia* spp. including *B. recurrentis* (9, 10, 69, 71, 103, 139, 161, 163, 170, 172, 179, 182, 191, 239, 248; D. Kaplan, Ph.D. thesis, Hebrew University, Jerusalem, Israel, 1944), the success of culture endeavors was usually dependent upon the quality of the animal serum or human ascitic fluid available or upon addition of a small amount of fresh blood to each culture. Kelly's formulation brought us closer to the goal of a fully defined medium (153, 154). Although Kelly's medium still contained serum, it and subsequent derivatives (23, 26, 98, 176, 236) have proved successful in several laboratories and reproducible in the yields they produce. (*B. burgdorferi* can grow in the absence of serum when a crude albumin preparation is used [41].) In these media, which rival or surpass mammalian cell culture media in their complexities, well-adapted strains of some *Borrelia* spp. have generation times of 6 to 12 h and reach cell densitities of 2×10^8 per ml (23, 26, 162). Cultures can be started with a single organism (23, 26, 235, 236).

One of the ingredients of Kelly's medium that aided growth and allowed higher cell densities to be obtained was *N*-acetylglucosamine; other amino sugars could not substitute for this compound in the medium (156). *N*-Acetylglucosamine is a major constituent of peptidoglycan, and we have found that labeled *N*-acetylglucosamine in the medium is incorporated into the insoluble cell wall fraction of borreliae (A. G. Barbour and R. Heiland, unpublished data). *N*-Acetylglucosamine is also, coincidentally or not, the primary building block for the chitin in a tick's cuticle (117).

Spirochetes generally prefer a viscous environment in which to swim by their screwlike motion (40). Gelatin, while not essential for growth, does permit higher cell densities to be achieved when starting with small inocula (156). Although some species tend to grow in clumps in broth medium, true colonies have as yet not been observed when borreliae are inoculated onto solid media (23, 248).

Antibiotic susceptibilities of *Borrelia* spp. are of interest not only for therapeutic reasons but also for the design of selective media and for insights into the physiology of this genus. Studies of the action of beta-lactam antibiotics have shown that these compounds affect cell wall synthesis and the integrity of the borrelial cell much as they do other eubacteria; *B. hermsii* has five penicillin-binding proteins (34). Borreliae are also susceptible to tetracyclines, chloramphenicol, and erythromycin (148, 147, 240). They are resistant, like some other spirochetes, to rifampin, sulfonamides, and 5-fluorouracil (1, 75, 122, 146, 231, 242). The phenotype of absolute rifampin resistance may indicate that a spirochete's RNA polymerase differs from those of other bacteria (198).

Increases in the resistance to antimicrobial agents have developed when borreliae were passed continuously in treated mice (90, 95, 121, 210). Usually the increases in minimal inhibitory concentrations were on the order of 10-fold or less (90, 95, 121, 210). Resistance to one class of antimicrobial agents was associated with the resistance to another class in some mutants (95, 121).

Borreliae can be passed several times in artificial media and yet still retain their infectivity for animals (182; Kaplan, Ph.D. thesis). However, the cultivated strains eventually lose this ability (236; Kaplan, Ph.D. thesis). The specific virulence traits that are no longer expressed have not been identified, but in one strain of *B. hermsii* loss of ability to produce relapsing disease was associated with change in molecular weight of the major surface protein (30, 32).

GENETICS

As might be supposed about a group of bacteria that cannot be grown as single colonies, divide in broth medium no more rapidly than every 6 h, and are class II biocontainment level pathogens, nothing was known for a long time of the DNA organization and the genetic systems of borreliae. What has been recently learned, though, is of interest and encourages further studies of these organisms.

Borreliae, like many other types of bacteria, have resident bacteriophage and plasmids (123, 138). Unique in borreliae, however, are the presence of extrachromosomal pieces of DNA that are linear (205). Linear plasmids have also been found in yeasts and one species of *Streptomyces* (98, 128). We assume that the ends of borrelial linear plasmids are "sealed," as they are in eucaryotic examples of linear DNA, through either a 5′-end-bound protein (98) or a hairpin structure (160). The role of the linear plasmids of *B. hermsii* in the antigenic variation manifested during relapsing fever is discussed below. The other plasmids in *Borrelia* spp. appear to be the traditional supercoiled variety (130; L. Mayer, C. Garon, and A. G. Barbour, unpublished observations).

Electron microscopy first revealed the presence of bacteriophage in a *Borrelia* sp. (123). In several cells of the B31 strain of *B. burgdorferi*, there were viruses with capsids similar in morphology to the B3 bacteriophages of *Caulobacter* spp. (219). Subsequently, we have found similar viruses associated with a strain of *B. hermsii* that has been passed in broth medium for several years in the laboratory (Fig. 6 and 7) (Hayes and Barbour, unpublished data). Previously, bacteriophage were reported in association with leptospires and *Treponema hyodysenteriae* (209, 213).

A resident virus which under certain conditions enters a lytic phase and lyses cultured cells may be the explanation of a phenomenon noted by early cultivators of borreliae. These investigators found that some cultures had a periodicity to them (9, 172, 179, 248). Following logarithmic growth of the culture, there was a sudden and steep reduction in the number of spirochetes present. This was usually followed by an increase in the number of cells in the medium again. The "lysis" was probably not, therefore, a direct result of nutrient depletion or build-up of toxic substances.

The failure of the restriction enzyme *Mbo*I but the successes of *Sau*3AI and *Dpn*I enzymes in digesting *B. hermsii* DNA indicates that this species has an adenine methylation

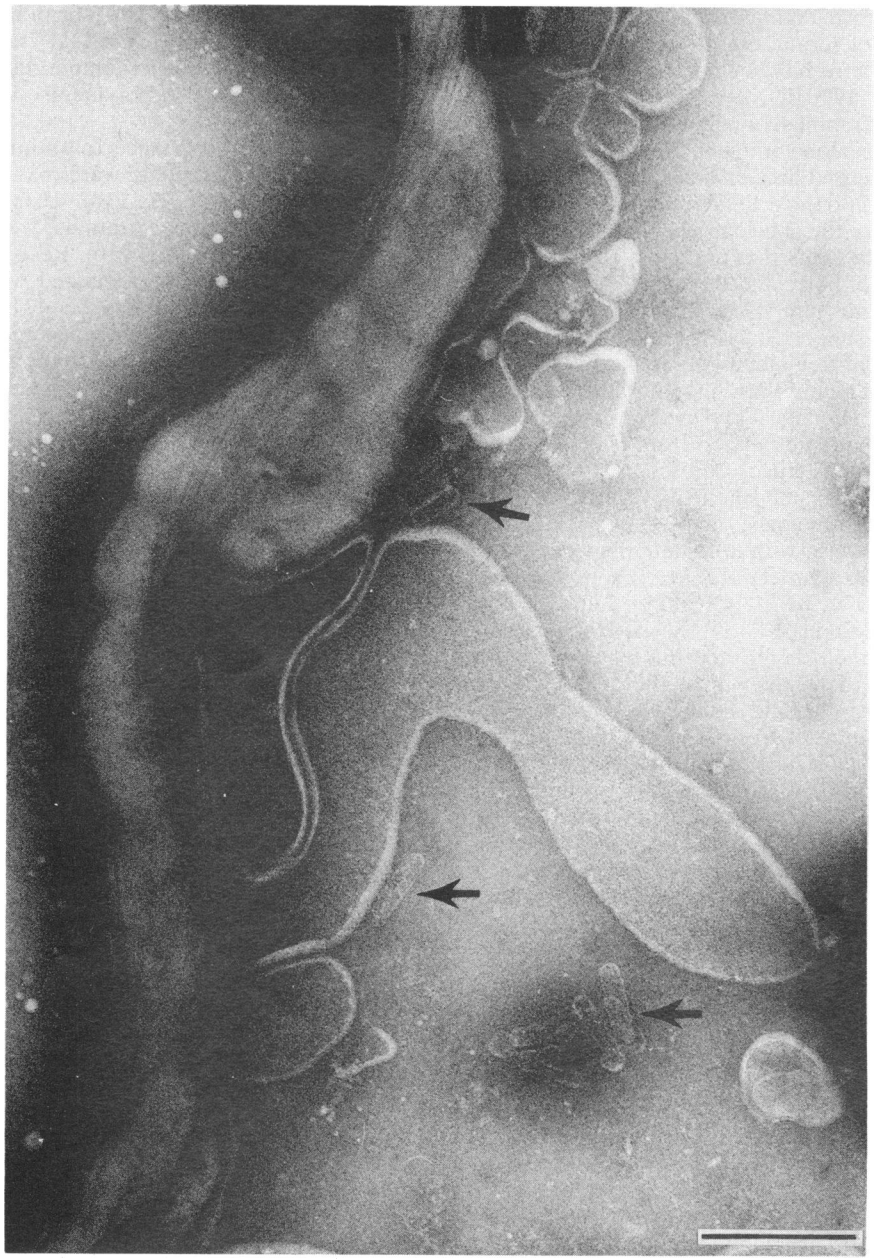

FIG. 6. Electron photmicrograph of a *B. hermsii* cell from a broth culture that was undergoing spontaneous lysis. Evident are numerous bacteriophage heads, some of which are indicated by arrows, and the disruption of the spirochete. The preparation was negatively stained with 2% ammonium molybdate. Bar, 0.2 μm.

system (183). Examination of other borreliae as well as other spirochetes for this trait may be useful for taxonomic purposes (22).

It is not known whether borreliae can exhange genetic information through conjugation, transformation, and transduction.

BORRELIA-HOST INTERACTIONS

We have chosen tick-borne relapsing fever *Borrelia* spp. as the principal examples of the relationship between borreliae and their hosts. In an operational sense ticks are the primary vectors of the disease-producing borreliae, but the generalized infections and transovarial transmission of borreliae in some ticks convince us that ticks should also be looked upon as infected hosts and studied as such with this bias in mind.

A comprehensive survey of the ecologic factors in *Borrelia* disease epidemiology and epizootology is beyond the scope of this review. It is realized, though, that ecologic and population-based approaches to *Borrelia* biology can lead to effective strategies for control or prevention of these arthropod-borne diseases.

As is metaphorically true for the chicken and egg, it may never be known whether borreliae were originally parasites or arthropods or of vertebrates. What we can appreciate now, though, is the importance (in a borrelia's anthropomorphic eyes) of both phyla for its and its progeny's ultimate

survival. Our tale then could start at any point in the cycle of transmission; we will arbitrarily begin with a tick feeding upon a spirochetemic vertebrate.

For most species of *Borrelia*, the usual vertebrate reservoir is a rodent (188). Some exceptions to this are the following: (i) *B. recurrentis* and *B. duttonii*, which apparently only utilize humans as a host (see below); (ii) *B. theileri* (49, 66, 227) and *B. coriaceae* (196), which are primarily associated with large animals such as cattle or deer; and (iii) *B. burgdorferi*, which is commonly found in deer as well as in mice and other rodents (6, 7, 47, 48, 177, 178; Anderson et al., in press; Bosler and Schulze, in press; R. S. Lane and W. Burgdorfer, Zentralbl. Bakteriol. Mikrobiol. Hyg., in press).

The large blood meal of the tick is held in the midgut where it is digested. The digestion of blood in ticks occurs intracellularly in the epithelium of the gut lining (5). This contrasts with the intraluminal digestion of blood by most hematophagous insects, such as the mosquito (5). As a consequence, borreliae in the tick midgut are not exposed to the proteases and acidity that they might encounter in an insect's midgut. A defense against proteases is probably not, therefore, required by borreliae for survival in ticks. *B. hermsii* and *B. burgdorferi* cell surfaces are, not unexpectedly, susceptible to attack by proteases, including trypsin (24, 31). The effect of proteases on *B. recurrentis*, which passes through the midgut of the louse (126, 127), an insect, has not been determined.

From this intestinal location and if the temperature is warm enough (94), most varieties of borreliae then penetrate the layers and membranes of the midgut and enter the hemocoel of the appropriate tick or louse (44, 56, 87, 93, 126). (An exception to this behavior may be *B. burgdorferi*, which is considered below.) In the hemolymph of this space, the borreliae multiply (56, 87, 116, 125, 127). The hemolymph is analogous to vertebrate blood and has a glucose and electrolyte composition not very different from mammalian serum (42). In lice the pH of the hemolymph was found to decrease from 7.7 to 6.9 during the course of borrelial infection (116); this was probably the result of lactic acid production.

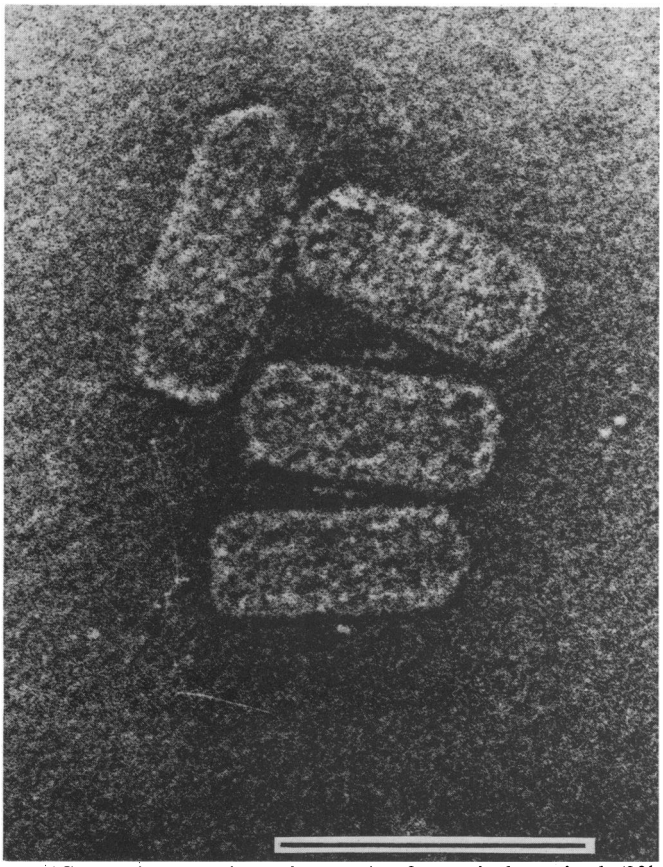

FIG. 7. Electron photomicrograph of negatively stained (2% ammonium molybdate) bacteriophage heads associated with *B. hermsii*. Bar, 0.1 μm.

As their numbers increase, the borreliae move toward certain organs in the tick and louse (56, 87, 93, 116). Burgdorfer (56), as well as others (116, 214), placed tick tissues in capillary tubes and observed the behavior of borreliae when the tubes were put in the spirochete-containing medium. The movements of the borreliae changed from being random with regard to orientation to characteristic of swimming behavior with a clear direction to it. The tissues acted as attractants to the borreliae. From measurements of the time interval between the introduction of the tissue and the "sensing" of the tissue by the borreliae, the size of the putative chemoattractant was calculated to be about that of an oligosaccharide (116). The borreliae were spoken of as having "tropisms" for certain organs.

In *O. moubata*, *A. persicus*, *Boophilus microplus*, and *O. coriaceus*, tissues that have high densities of spirochetes are the ganglia, salivary glands, and reproductive organs (56, 87, 171, 226, 243). Although the borreliae are primarily extracellular in these organs, there have been demonstrations of spirochetes within tick cells (4). Spirochetes are often found, usually in a state of disintegration, within hemocytes, the professional phagocytes of the hemolymph (126). In *B. microplus*, masses of *B. theileri* organisms were found associated with the surface of hemocytes (226).

The association of borreliae with ganglia or other nerve tissue has also been noted in other tick species (102) and in lice (116, 126). The propensity for borreliae to go to the brain of infected mammals (see below) suggests that the relationship between these spirochetes and neural tissues is not trivial. Further study of this attraction and the interaction that follows may reveal the basis for the significant nerve and brain involvement in Lyme borreliosis (201, 206, 233; R. Ackermann, B. Rehse-Kupper, and E. Gollmer, Zentralbl. Bakteriol. Mikrobiol. Hyg., in press; A. R. Pachner and A. C. Steere, Zentralbl. Bakteriol. Mikrobiol. Hyg. Ser. A, in press).

Borreliae are transmitted transovarially in many species of ticks: examples are *B. duttonii* by *O. moubata* (3, 46) and *B. anserina* by *A. persicus* (250). The borreliae in the ovary invade the developing oocyte-yolk complex from the hemolymph before the impervious shell forms around the egg (3, 46). During embryonic development spirochetes migrate from the yolk region to ganglia (3). The proportion of females that deposit infected eggs may be 80% or higher (transovarial infection rate), and 80% or more of the larvae that emerge from these eggs may be infected with borreliae (filial infection rate; 3, 63, 226, 250). In some cases the larvae of the next generation may not be capable of transmitting the borreliae, but in these instances the nymphs after they feed usually are transmitters (189, 200). Transovarial transmission can be very efficient, and passages over five to nine generations of ticks have been documented in the laboratory (16). For some, but not all, of the borrelia sublines that have been passed in ticks this way there has been loss of infectivity for laboratory animals (16, 105, 107). A specific viru-

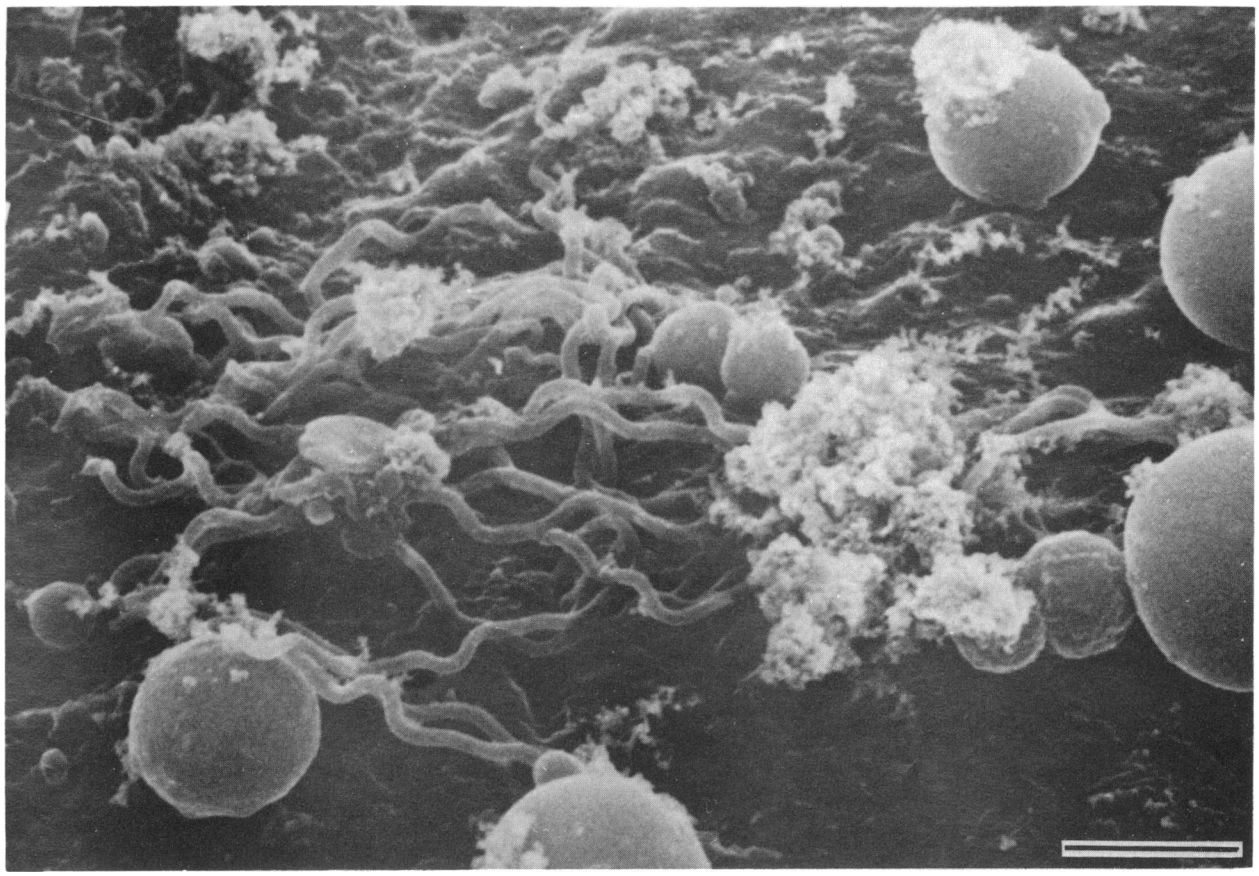

FIG. 8. Scanning electron microscope picture of *B. burgdorferi* spirochetes associated with the epithelium of the midgut of an *I. dammini* tick. Bar, 2.0 μm. (Photograph courtesy of D. Corwin, Rocky Mountain Laboratories.)

lence factor that is important in initiating infections of mammals may have been lost during passage in ticks. Change in antigenic serotype of a strain during tick passage was documented with polyclonal antisera (167). The continued vigor of a *Borrelia* species may depend upon periodic passage back into mammalian hosts. If this is true, it is not inconsequential for disease control programs: reduction of the number of susceptible hosts for a tick species, e.g., *O. moubata* through improved housing in villages, could lead to selection of strains of a borrelia that are less infectious for mammals. Such an outcome was postulated by Geigy et al. (107).

The testes of ticks may also be infected, and male-to-female venereal transmission has been documented. It was rare in *O. moubata* infected with *B. duttonii* (110, 245) but more common in *O. erraticus* containing *B. crocidurae* (101). The significance of venereal transfer and the horizontal spread of borreliae within tick populations remains to be determined.

Invasion of the salivary glands or the coxal organs is critical for transmission of tick-borne relapsing fever borreliae to a mammalian host. The coxal organs in the argasid, i.e., soft-shelled ticks, such as the *Ornithodoros* species, are specialized tissues for excretion of the excess fluids and solutes that accumulate in the tick during feeding (151). In the case of argasid ticks, like *O. moubata*, from which the coxal fluid is released near the mouth parts during feeding, presence of spirochetes in the coxal fluid is important for transmission (44, 243). Borreliae have the ability to actively penetrate from the hemocoel into the coxal organ and its fluid (45).

The coxal fluid route is not important, though, for argasid ticks that excrete coxal fluid after feeding. In these argasid ticks and in the ixodid, i.e., hard-shelled ticks, which do not have a coxal organ and instead depend upon the salivary glands for fluid volume regulation (151), transmission can occur via the saliva (102, 243).

Another method of transmission between tick and vertebrate appears to operate in *Ixodes dammini* ticks infected with *B. burgdorferi*. In this relationship, the borreliae are distributed primarily in the midgut and occasionally in the hindgut and rectal area; in the majority of ticks no other tissues were observed to harbor spirochetes (58, 62). Figure 8 shows *B. burgdorferi* cells in close apposition to the microvillar brush border of the gut epithelium. The accompanying figure (Fig. 9) shows penetration and colonization of the gut wall adjacent to the basal lamina. From this intestinal residence the borreliae may be transmitted to a vertebrate host by the regurgitation of gut contents during the feeding act (58).

The louse provides another route of transmission. The bite of the louse itself is not the source of borreliae, and neither are the feces (73). Rather, transmission occurs when the infested person crushes the irritating louse and inadvertently rubs the spirochete-rich hemolymph into the bite wound or carries on the fingers contaminated fluid to the conjuctiva (73).

It should also be mentioned in regard to the arthropod

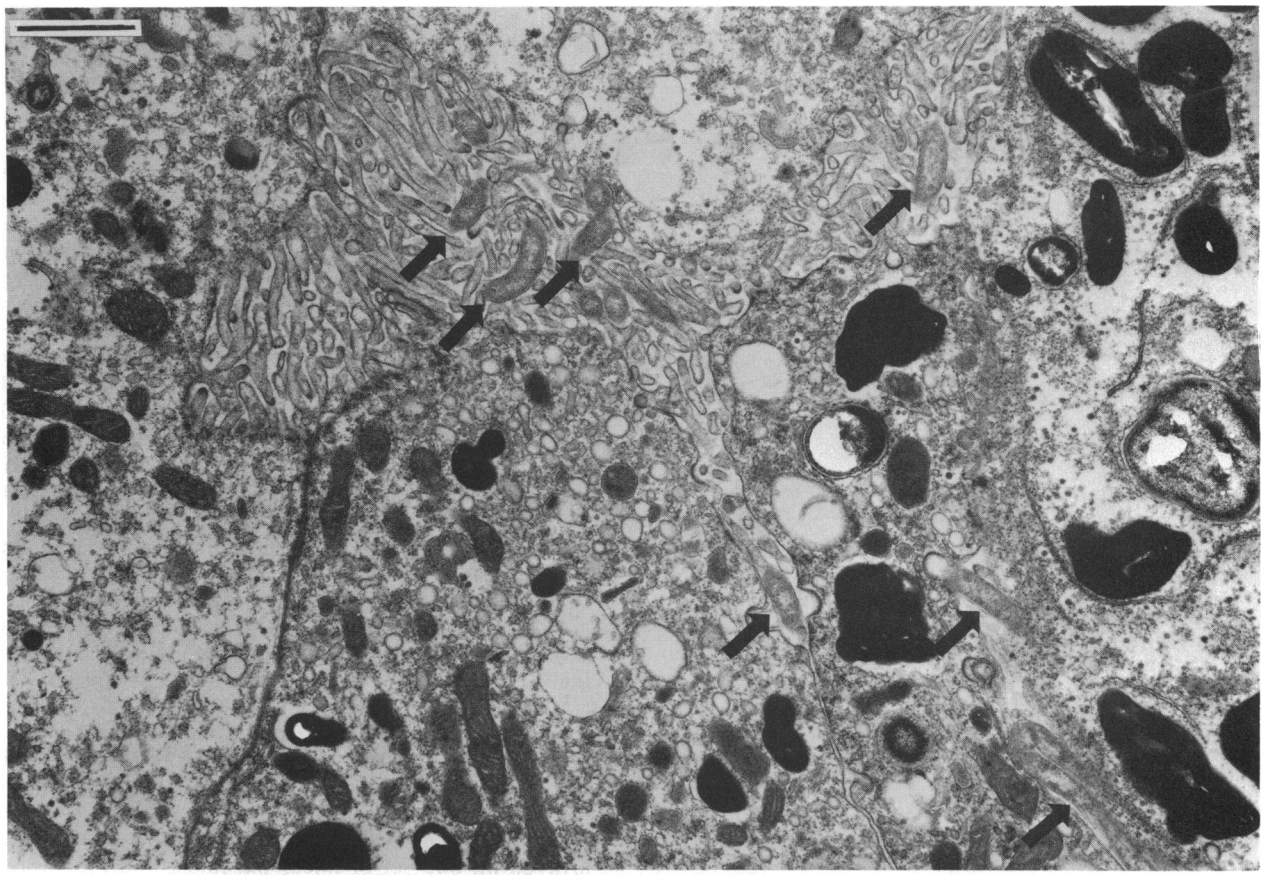

FIG. 9. Electron photomicrograph of thin section of midgut of an *I. dammini* tick infected with *B. burgdorferi*. Numerous spirochetes, indicated by arrows, can be seen in close apposition to the villar processes of the midgut wall and in the deep intercellular spaces of the wall. Bar, 1.0 μm.

vector that ticks, particularly the soft-shelled varieties, are notable for the long periods of starvation they can endure. There are examples of ticks going without a blood meal for 6 to 7 years and yet remaining infectious (97, 199). During starvation the borreliae tend to disappear from the hemolymph but persist in the organs (109).

A single borrelia of *B. turicatae*, *B. hermsii*, or *B. duttonii* is sufficient to produce infection of laboratory animals (108, 221, 236). Experimentally, the minimal infective inoculum was lower for the interperitoneal route than for the subcutaneous route (89). Borreliae appear in the blood as soon as 1 h after intraperitoneal inoculation of a large inoculum (89). Once in the blood the relapsing fever borreliae multiply as often as once every 6 h (89, 236). In susceptible animals, there may be as many as 10,000,000 borreliae per ml of blood during peak spirochetemias (70, 236). Borrelia burdens in the blood of fowls infected with *B. anserina* can also be heavy (21). In contrast, *B. burgdorferi* spirochetemias are light in feral animals and experimental animals (38, 48, 59, 145, 164; G. Stanek, I. Burger, A. Hirschl, G. Wewalka, and A. Radda, Zentralbl. Bakteriol. Mikrobiol. Hyg. Ser. A). Spirochetes are detectable in the blood of cattle and horses with *B. theileri* infections, but the counts are not high (49, 66, 227). In light infections of the blood and in the periods between relapses only 10 to 1,000 borreliae per ml may be circulating (70, 236). In the case of the relapsing fever borreliae, these few organisms can be detected by inoculating susceptible, nonimmune animals (70, 236).

The serum resistance of the *Borrelia* spp. may be a determinant of its vertebrate host range. For example, *B. duttoni* survived in vitro in fresh sera from mice, guinea pigs, rabbits, chickens, and horses, but not in bovine, goat, sheep, or pig sera (77).

The relapsing borreliae circulate and multiply in the blood until specific antibody appears. Once the concentration of antibody is high enough, the organisms rapidly disappear from the blood. After T-cell-independent antibodies bind (186), clearance of borreliae is probably mediated by either complement-induced lysis or phagocytes with C3b receptors (185). The immune response to relapsing fever borreliae is reviewed in more detail elsewhere (A. G. Barbour, Contrib. Microbiol. Immunol., in press). Determinants of the antibody-mediated clearance of Lyme disease borreliae are under investigation.

When relapsing fever borreliae are no longer detectable in the blood, they may still be found in organs (120). Although borreliae can usually be recovered from such organs as the spleen, liver, kidneys, and eyes of infected animals (37, 120), the organ usually with the most persistent infections is the brain. Humans with relapsing fever have had borreliae recovered from the cerebrospinal fluid (72). Borreliae can be recovered from the brains of animals that are immune to challenge with that strain (119, 127, 148, 178). Detection or isolation of borreliae from brains of animals that had been infected several months and up to 3 years previously has been reported (12, 181, 197, 223). Before the advent of modern ultracold freezers, strains were kept in the brains of rodents and passed once or twice a year (92). Some species

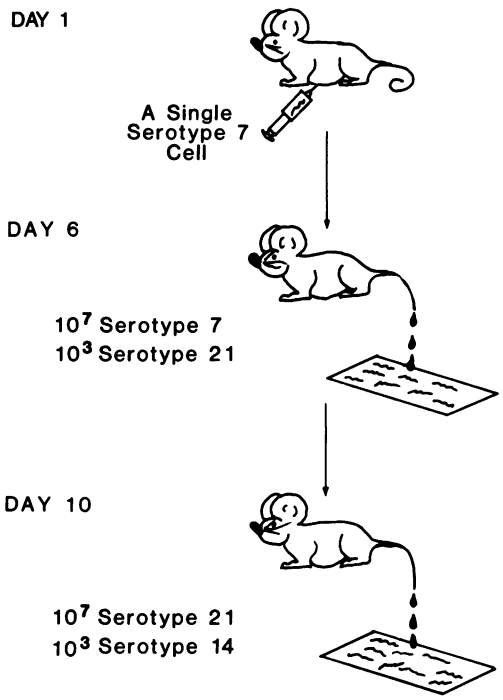

FIG. 10. Representation of antigenic variation during infection with a relapsing fever *Borrelia* sp. (30 and 236). On day 1 a mouse is injected intraperitoneally with a single cell of serotype 7 of *B. hermsii* HS1. By day 6 there are approximately 10^7 serotype 7 borreliae per ml of mouse blood; in addition, there are 10^3 cells of a new serotype, 21. Between days 6 and 10 the original serotype, 7, is cleared from the blood of the mouse by neutralizing antibodies produced in response to the first wave of spirochetes. On day 10 serotype 21 is now the predominant serotype; this peak in the population corresponds to the first relapse. Serotype 14 cells are present in low numbers at this time, but will be, after the host clears the serotype 21 cells, the predominant serotype in the second relapse. The serotypes in the mouse blood, which is obtained from the tail vein, are determined by indirect immunofluorescence with serotype-specific monoclonal antibodies (32, 35).

and even particular strains within one species were found to be more "neurotropic" than others (166). This designation was made if a high percentage of animals had residual brain involvement and the borreliae persisted in the brain. In all organs the borreliae are usually extracellular. Similar tissue distributions of organisms have been reported with *B. anserina* (21, 140) and *B. burgdorferi* (145, 176; Anderson et al., in press, P. H. Duray and R. C. Johnson, Proc. Soc. Exp. Biol. Med., in press).

Although ingestion of spirochetemic blood by an arthropod and subsequent passage of the borreliae to another vertebrate host as outlined above is the usual mode of transfer between animals, direct vertebrate-to-vertebrate transmission may also occur. The urine of infected animals can contain viable borrelia (Bosler and Schulze, in press). Spirochetes in the urine could enter the host through the mucous membranes of the conjunctiva, mouth, or nose (73). Borreliae were demonstrated in the milk, and a small proportion of guinea pigs that consumed milk of an infected female became infected themselves (222). The demonstration that rats and dogs can be infected through the consumption of infected rat brains or other infected organs (132, 149, 158, 215) suggests that a selective advantage could be conferred upon those strains that are neurotropic and that infect rodents practicing some degree of cannibalism. Rat saliva has been found to be infectious, and transmission through rat bites has been reported (131). Contact transmission of *B. burgdorferi* among laboratory-housed field mice has been documented, but the route of transmission was not known (64).

In relapsing fever, the number of days during which borreliae circulate, therefore being available for vectorial transmission, is increased by the parasitic strategy of antigenic variation (79, 184, 236; reviewed in Barbour, in press). The 10 to 1,000 borreliae circulating between relapses are not representative of the predominant serotype of the previous relapse but instead are examples of a new serotype which is soon to cause the next relapse (Fig. 10) (30, 236). In *B. hermsii* the VMP proteins described above are the determiners of serotype specificity; there appears to be a different VMP gene for each of the 25 or so different VMP proteins (183, 205). New serotypes arise spontaneously in a population of *B. hermsii* at a frequency of approximately 10^{-4} to 10^{-3} per cell per generation (236).

The genetic mechanism that brings about a change in surface VMP, and thus a change in serotype is transposition of a copy of a VMP gene from a silent (nonexpression) to an active (expression) locus (183, 205). In the process of this transposition the old VMP gene sitting at the expression locus is displaced by the new incoming VMP gene. The old VMP gene encoded the surface antigen of the infecting serotype. The new VMP gene encodes a different VMP protein and one that represents the serotype prevalent in the host during the relapse. In cells of the two serotypes of *B. hermsii* strain HS1 that have been studied in most detail, the silent copies of the two serotype-specific VMP genes are arrayed on one set of linear plasmids, and the expression locus and the active copy of the VMP gene are located on different linear plasmids in the cell (205).

B. anserina does not appear to undergo antigenic variation: the infected birds do not have discernible relapses of illness (21). However, the antigens of this species have not been studied in enough detail to allow precise determination of the antigens that are expressed at any given time in an infected animal.

Studies of *B. burgdorferi* still suffer from a lack of an experimental animal model that has easily detectable spirochetemias. Therefore, at this time we cannot emphatically say whether or not antigenic variation occurs in this species.

Borrelia spp. that are long passaged in mice or other animals may lose the ability to infect and be transmitted by ticks (51, 113). In one such strain of *B. duttonii* that was examined, the borreliae were able to persist in the midgut of *O. moubata* after feeding, but they could not enter the hemocoel (244). However, when the *B. duttonii* organisms were injected directly into the hemolymph in the hemocoel, ticks were then capable of transmitting the borreliae to mice (244). It appears that the mouse-passaged borrelial variants were no longer capable of penetrating the tick's midgut wall. Long-term animal passage need not always result in loss of the ability to be transmitted by ticks; a strain of *B. hispanica* was passed 71 times in guinea pigs and yet remained tick transmissible (75).

A strain of *B. duttonii* that had been passed many times in mice was found to have lost virulence for humans (212). When using borreliae for pyrotherapy of neurosyphilis, the authors of this report recommended that no more than 30 to 40 passages in mice be made before inoculation of the strain back into humans (212).

ACKNOWLEDGMENTS

We thank Betty Kester and Irene Rodriguez for preparation of the manuscript, Liza Hamby for library research, and Willy Burgdorfer for his very helpful comments and for carrying the torch of "borreliology."

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