I

HUMAN BORRELIOSIS (RELAPSING FEVER)

RELAPSING FEVER (borreliosis) may appear either in louse-borne epidemics or in sporadic tick-borne instances. The disease is characterized by recurring attacks of fever, usually of decreasing intensity and duration. Relapsing fever, however, may be of sufficient intensity as to terminate in death.

The disease has been named "febris recurrens," "recurrent fever," "famine fever," "spirillum fever," "spirochetal fever," "vagabond fever" (Spain), "fowl nest fever" (China), "gharib gez" (Iran), "Giesinger's bilious typhoid" (Egypt), "carapata" (Africa), "kimputu," "gorgoya" (South America), "tick fever." It has also been given other *epitheton ornans* -es taken from local picturesque descriptive designations and from more or less fortunate combinations of greco-neo-latin terms. The generally accepted name, however, is relapsing fever and, in countries adhering to Latin nomenclature, "febris recurrens."

The causative agent, *Borrelia,* is insect-borne and is transferred from man to man directly only under unusual circumstances. The disease acquires epidemiologic importance principally among people who are compelled to live under unfavorable hygienic conditions. The epidemiologic aspects of endemic or tick-borne relapsing fever that is carried by some species of *Ornithodoros* depend upon the interrelationship of man and ticks, and often also on a mammalian host. There are challenging features and many little known aspects of this disease. The widespread relapsing fever outbreaks

that developed during the end of and after World War II, the constant occurrence of the infection in Africa and Asia, and the apparent hitherto confused picture of vectors and agents in Central and South America have not as yet stimulated many researchers

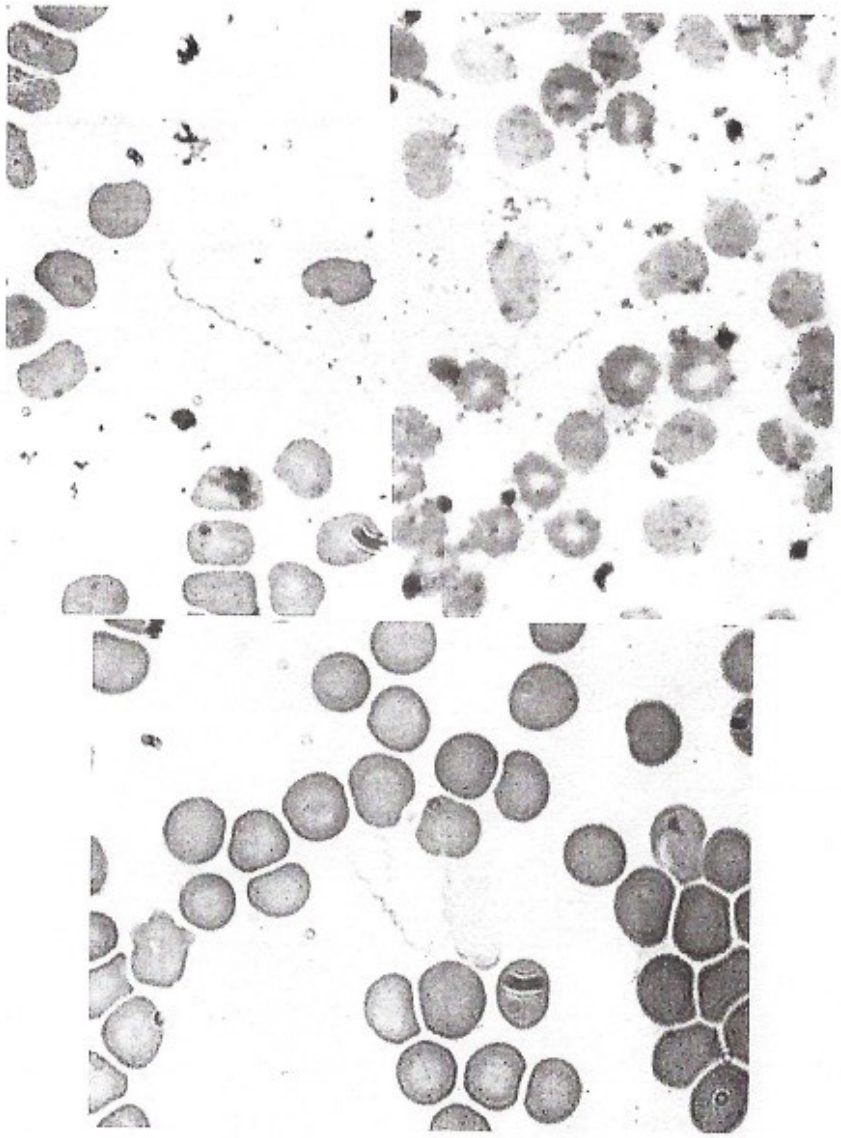

FIGURE 1. *Borrelia* in blood. Giemsa stain. x 950.

to delve into this problem. Neither have some interesting observations made during and after World War II fully penetrated into the world literature. While important basic research on *Borrelia* and its vectors is being performed in several institutions, the influence of the host-parasite relationship on the epidemiology of relapsing fever and the course of the disease in individual patients are stressed only in a few textbooks. It seemed desirable, therefore, to survey the literature on these aspects of relapsing fever, and to present such knowledge in a critical review which may assist scientists who wish to approach this manifold problem.

Several chapters in textbooks and review articles deal with more than one aspect of relapsing fever, as those of Hindle (363, 364, 365), Mühlens (509), Walters (709), Simmons (647), Geigy and Herbig (308), Mooser (500), Felsenfeld (264), Whitmore (731, 732), the Symposium on Relapsing Fever in the Americas in 1942, sponsored by the American Association for the Advancement of Science, and others. Geigy (303) summarized his long experience with relapsing fever in Africa, and Southern and Sanford (659) recently surveyed pertinent data on relapsing fever. Other reviews, dealing with specific problems of borreliosis, will be noted in the respective chapters.

HISTORY

Many historical accounts of relapsing fever are based on the studies of Scott (634).

Epidemic relapsing fever was recorded and described by Hippocrates in Thasos as "ardent fever" (308, 634). Bryceson *et al.* (127) believe that the "yellow fever" experienced in Europe during the VIth century may have been relapsing fever. The five epidemics of "sweating sickness" that swept England between 1485 and 1551 included outbreaks of relapsing fever (127, 491). Louse-borne typhus also prevailed at that time and "famine fever" was the common designation of both infections. Gloucestershire was wiped out in the beginning of the XVIIIth century by "famine fever." England and Ireland suffered from this disease also in the XVIIth and XVIIIth centuries. The first well-documented epidemic of relapsing fever was described in Ireland between 1739 and 1741 when Rutty recorded its clinical features. The epidemic then spread

from Ireland to Scotland and England. The outbreak in 1834-1848 in Edinburg was particularly severe. The designation "relapsing fever" was first used by Craigie in 1843.

The Scandinavian countries became infected in 1788 from Russian ships that made port in Sweden. The last epidemic in Germany occurred from 1867 to 1868, in Ireland from 1868 to 1871. Later, improving hygienic conditions kept the disease from West Europe.

Louse-borne relapsing fever was imported into the United States from England and in 1844 caused the famous Philadelphia epidemic. This infection persisted in the eastern region of the United States for about 30 years. In 1874, there was a similar outbreak among Chinese laborers in California (193). Tick-borne relapsing fever was recognized soon after the West was settled (727).

The causative agent of epidemic relapsing fever was discovered in 1868 by Obermeier who did not publish his paper on this subject, however, until 1873 (507). This organism is now designated as *Borrelia recurrentis*. In the past, it was called *Spirocheta obermeieri, Protomycetum recurrentis, Spirocheta recurrentis,* and other names (128).

Little is known about louse-borne relapsing fever before the XVIIIth century except that it prevailed in cold and poor countries where lice were common. It was known to have occurred in Africa and in China (149, 363, 641).

Carlisle (145), Geigy (303), and others agree that Livingstone was the first to note in 1857 that tick-borne relapsing fever was present in Angola and Mozambique and that the disease was familiar to the local African and Portuguese inhabitants. Lamoureaux (430) reported that Drury had observed tick-borne relapsing fever during his trip to Madagascar in 1702-1720 but it remained for Cook (198) in Uganda to notice the presence of *Borrelia* in the blood. Ross and Milne (613) in the same area and simultaneously Dutton and Todd (250) in the Congoes, as well as Koch (417) in East Africa, confirmed these findings and demonstrated that tick-borne *Borrelia* may cause disease in monkeys and man. Both Dutton and Todd contracted the disease, and Dutton died of it (303).

The "bilious typhoid" in Egypt, described by Griesinger in 1857 (617) appears to have been relapsing fever, but Napoleon's surgeons were the first to diagnose relapsing fever correctly in that country (395).

Among other investigations of historical importance are those of Koch (418) in East Africa which resulted in the naming of a *Borrelia* strain *Spirochaeta kochi;* the studies of Novy and Knapp (547) of the movement of *B. recurrentis,* and their observation that antibodies begin to develop during the first attack of the disease; the review of Hindle (361) of relapsing fever in tropical Africa; the investigation of the mode of division of borreliae and a review of the *Borrelia* problem by Dobell (243); the experiments of Ross (612) on patas monkeys with strains isolated in Uganda; the survey of the distribution of the vector *(Ornithodoros moubata)* of *B. duttonii* in Africa by Nuttall (548); the attempts of Nicolle *et al.* (536) to transmit tick-borne borreliae to lice; the first demonstration of the infectiousness of the coxal fluid of *O. moubata* by Todd (684); and the study of the ecology of *O. moubata* between 1892 to 1905 by Todd (685) which led him to predict that as travel increased, the tick, and with it also tick-borne relapsing fever, would spread further through Africa.

Relapsing fever has been observed in all parts of the world, except Australia, New Zealand, and Oceania, where only solitary imported cases have been seen (363).

In Africa, reports of past occurrences are available from the former Gold Coast (617, 618). LeGac (439) described an epidemic in Oudaii (Chad) between 1925-1928. Hawkins (338) studied *B. duttonii* infections in Tanganyika. Fendall and Grounds (269) believed the disease to be retreating in Kenya, where louse-borne relapsing fever was introduced by refugees from Abyssinia (296). The Sudan had several louse-borne outbreaks. After the first world war, repatriates brought infected lice with them to that country. The ensuing epidemic stopped only at the forest (439). Atkey (30) described this 1926-1928 outbreak. Kirk (411) believed that the constant population movement from and to Abyssinia steadily supplies the Sudan with infected lice. Hindle (365) observed that louse-borne relapsing fever swept over the major part of the northern part of Africa during 1925-1928.

Relapsing fever is not new to many parts of Africa. Kirk (411) noted the louse-borne relapsing fever was introduced into the Sudan from Egypt between 1908 and 1924, in 1926 from French West Africa, and during the Abyssinian War in 1936 from Italian Africa.

Egypt suffered during the Napoleonic invasion (see above) and, according to Kamal *et al.* (395), experienced an outbreak that was recognized as relapsing fever by Sandwirth and Engel in 1884. The disease has been systematically studied since 1906 in that country.

Algeria, Tunisia, and Morocco have been recording louse-borne relapsing fever since 1903 (534, 535, 641, 642). The 1907-1914 epidemic in Algeria offered an occasion for numerous scientific studies.

Somaliland, closely adjacent to the Abyssinian-Sudanese focus of relapsing fever, experienced tick-borne cases among troops in her formerly British territory (244). Other occurrences of the disease were recorded by Italian authors (610). Moise (497) commented on relapsing fever in Somaliland, pointing out that the most effective African tick vector, *O. moubata,* is very scarce in that area. He suspected that there were other transmitting agents. A similar dispute arose in Madagascar, where Lamoureaux (430) recorded the appearance of the disease in military personnel who used the route between Morandava and Majuna in 1911 and 1912. Suldey (672) encountered tick-borne cases on the west coast of Madagascar in spite of the scarcity of a recognized vector.

In South Africa, relapsing fever remained undiagnosed until an outbreak was observed in the Cape Province among miners who had come from the North and had brought with them the tick *O. moubata* as a good-luck charm which then began to thrive in their huts.

In Yemen, in Asia Minor, Franchini (277) was unable to find ticks that were capable of conveying borreliae and concluded that the louse was the only vector.

During the first world war, Egypt was heavily hit by louse-borne relapsing fever (395, 670). In the four years between 1916 and 1920, 40,000 cases were recorded. Syria and Turkey also became infested (628, 734). Mesopotamia had an outbreak in 1918 among Turkish railroad workers (519). During the aftermath of World War I, from February to August 1920, troops became infected in Birjand, East Persia. Relapsing fever in the absence of lice was reported in Jinnuk, East Persia (337, 739), while the areas of Sharifabad, Meshed, and Turbad had ticks as well as human lice that were able to carry borreliae.

In India, where Mackie was the first to state in 1907 that the human louse carried epidemic relapsing fever, numerous episodes of the disease were recorded. "Spirillar fever" was described in a group of Ghurka tea garden workers in Darjeeling (392). Roy (614) reported an outbreak in the Seoni District (Upper Provinces) in 1919-1920. It was believed (206, 207) that louse-borne relapsing fever was endemic in Agra and Oudh, and spread from there to other areas. Gill (316) published the statistics on the outbreaks in the Punjab in 1869, 1878, 1891, 1906, and 1920 which claimed 26,000 lives.

Browse (114) described tick-borne recurrent fever in Qetta, principally among military camp followers who were new to the area and who lacked immunity.

Cases were described in Afghanistan by Avanessov (33) who did not, however, find the vector.

China has been the home of louse-borne relapsing fever for a long time. Records in the western literature are available from West China (387), Sechuan (388), an outbreak in an oprhanage in Peiping (172), and a review of 337 cases treated in the Peiping University Medical College Hospital between 1921-1937 (176). Toyoda (687) studied *Borrelia* strains collected in Manchuria.

Indochina was infected from China. There were 373 cases in Hanoi during the first half of 1912 (508). The disease was found in Nghê-An (357). Casoux (149) and Millous (495) described the course of relapsing fever that during the 1910-1911 cool period alone took about 500 lives in Annam. The disease spilled over into Thailand (733). Further data are not available from that country.

As stated previously, louse-borne relapsing fever came from China to the United States in 1874 (494). Later, however, tick-borne relapsing fever was the type of the disease that prevailed on the Pacific Coast. A summary of such instances for the years 1921-1937 was presented by Wheeler (737). Meador (489) reported 5 cases from Colorado. Bannister (66), Hemingway *et al.* (356) and Thayer (680) rendered further reports from the Southwest and Northwest. Graham (321) described a case of a boy who entered a cave in Denton County, Texas, that has been known for years to harbor infested ticks, and became ill with relapsing

fever. Kemp *et al.* (408) surveyed the epidemiology in Texas. Closson (183) described it in Kansas. Briggs (111) and Coleman (193, 194) reviewed the situation in California. Palmer and Crawford (560) reported an outbreak in British Columbia. The transmitting tick was not found in that episode in Canada.

Louse-borne relapsing fever was observed in Panama by Connor (197). Tick-borne cases were seen in March and April, 1921, in hunters and in rats captured in the Arraiján area (68). Dunn and Clark (248) studied animal hosts and vectors in Panama.

Relapsing fever was introduced to Ecuador probably from Colombia during the revolution in 1896 (443). It is now tick-borne in both countries.

Chiriboga (164) reported louse-borne infections from Peru. Prado (588, 589) described the history of the disease in that country, dating back to population movements in the late XIXth century.

During the Balkan War louse-borne relapsing fever was not common in Macedonia in spite of the presence of the vector. The disease was at home in the trenches of World War I, principally in the countries of the Central Powers and in East Europe. An outbreak initiated by the distribution of Austrian prisoners of war in Serbia for work in the fields lasted 6 months (377). Cantacuzène (143) noted that louse-borne relapsing fever was endemic in the eastern part (Besarabia) of Rumania but there was an upsurge of the disease and of epidemic typhus in 1916. Poland was heavily infested at that time. The city of Lodz alone had 343 cases between March and December, 1917 (473). Sterling-Okunewski (669) and Lipinski (453) reported additional instances in Poland and in Hungary. Oettinger and Helbreich (551) related the course of the disease on the Russian front.

The tick-borne form has been observed in the Caucasus since 1928 by Maruashvilli (475) and in Tashkent (398). A thorough analysis of the natural foci of the disease in Turkemenia followed later by Petrishcheva (571).

It appears that the association of man and louse that was considered a somewhat vexing event but not an evil part of life, until hygienic concepts began to prevail in the 19th and 20th centuries, has endowed the history of louse-borne relapsing fever with a con-

tinuity that has been interrupted by epidemic outbursts perhaps only at times when the immunity of larger population groups reached a low ebb, or when hitherto uninfected populations came into contact with infected groups of people.

Louse-borne relapsing fever spread during and after World War I over large areas of the Old World as a result of the propagation of lice among dislocated soldiers and civilians living under unhygienic conditions, who were compelled to move about from locale to locale. These epidemics gave impetus to the study of the disease. Most basic clinical and epidemiologic concepts of relapsing fever were elaborated during these periods. Further outbreaks, as well as less extensive epidemics, have also been studied thoroughly and will be analyzed in subsequent chapters. Among these belongs the recognition of the continuity and contiguity of louse-borne relapsing fever.

The study of tick-borne or endemic borreliosis went through the same phase as the rest of microbiology, when the major interest apparently was to discover new species and strains. Borreliosis is a dynamic condition, a cyclic disease characterized by a cyclic causative agent. No wonder that great numbers of species have been proposed on the basis of minor or not fully understood strain variations and mutations, incomplete knowledge of the vector, and epidemiologic observations that were not extended far enough "longitudinally" and "laterally." Some of the taxonomy both of *Borrelia* and *Ornithodoros* still has to be untangled, principally in Central Asia and South America. A further complication arises from the transliteration of the names of some authors, principally in the U.S.S.R., who had published in Russian and German, choosing the method of transcription of certain letters of the Cyrillic alphabet more or less arbitrarily. One encounters in the literature Pavlovsky, Pawlowski, Pavlovskii, Pavlovskyi; *B. latishevyi, B. latischewyi, B. latishewyi,* and so forth. The Reader is asked for his kind indulgence when encountering such discrepancies, principally in the references.

CAUSATIVE AGENT

Taxonomy

Detailed discussions of the taxonomy and biology of *Borrelia* have been presented by Baltazard *et. al.* (62), Davis (222, 223),

Dobell (243), Geigy (303), Geigy and Herbig (308), Geiman (314), Gelman (315), Heisch (342, 343), Hindle (363, 364, 365), Johnstone (386), Moursund (507), Nicolle and Anderson (521, 526), Schuhardt (630), Walters (709), and others.

The common *Borrelia* strains that cause relapsing fever in man are listed in Table 1, together with their vectors and geographic distribution.

The genus *Borrelia* Swellengrebel 1907 is a member of the family Treponemataceae Robinson 1948 which belongs in the order Spirochaetales Buchanan 1918. Until recent electron microscopic

TABLE 1
BORRELIAE CAUSING HUMAN RELAPSING FEVER, THEIR VECTORS AND GEOGRAPHICAL DISTRIBUTION

Borrelia	*Vector*	*Geographical Distribution*
B. recurrentis	*Pediculus humanus*	Potentially cosmopolitan
B. hispanica	*Ornithodoros erraticus erraticus*	Mediterranean, Middle East, East and West Africa, from Uganda to Iran
crocidurae group*	*O. erraticus sonrai*	
B. crocidurae		Middle East, Central Africa
B. microti		Middle East
B. merionesi		West Africa
B. dipodilli		East Africa
B. duttonii	*O. moubata*	Principally East and South Africa
B. persica	*O. tholozani* (O. papillipes)	Eastern Mediterranean, Arab Peninsula, Iran, Central Asia
B. latyschewii	*O. tartakovskyi*	Central Asia
B. caucasica	*O. verrucosus*	Caucasus
B. venezolensis	*O. rudis* (O.venezolensis)	Northern part of South America
unnamed (dugesii?)	*O. talaje*	Central and South America, Western US, Canada
B. mazzottii	*O. talaje* (substrain?)	Central, possibly also South Amercia, Texas
B. turicatae	*O. turicata*	Central and South America, Western US, Canada
B. parkerii	*O. parkeri*	Western US, Canada
B. hermsii	*O. hermsi*	Western US

* Human pathogenicity low.

and some biochemical studies were completed, members of the genus *Borrelia* were described merely as unicellular, spiral organisms without a rigid cell wall, with broad and irregular, loose spirals of inconstant amplitude, motile by an axial filament, easily stained with aniline dyes but difficult to cultivate on artificial media, parasitic to man and animals, and principally propagated by insects (102, 132).

Borreliae have been designated by a number of different names in the past, such as *Protomycetum, Spirochaeta, Spirocheta, Spirillum, Spironema, Treponema* etc. The European literature, including that of the U.S.S.R., still uses *Spirochaeta* in certain publications whereas this term should be reserved only for free-living forms.

The identification of *Borrelia* species according to the usual bacteriologic characteristics is difficult if not impossible. The organisms are not easily cultured, and their antigenic phase variations during relapses, which is one of the principal features of the agent, often preclude serologic diagnosis. Animal responses may be variable. The morphologic characteristics of all recognized species are about the same (128, 132) and often depend on the fixative and staining method employed (46, 47, 196 and others). This is particularly true when silver impregnation methods are used (386). Other means of classification had to be sought and were found in the agent-vector relationship.

B. recurrentis, the cause of epidemic relapsing fever, was originally designated *Protomycetum recurrentis* by Lebert in 1874; *Spirochaeta obermeieri* by Cohn in 1875; and *Treponema recurrentis* by Schaudinn in 1905, before it was classified as a member of the genus *Borrelia.* Some of its sub-strains will be discussed later.

The primarily tick-borne borreliae have been classified by Geigy (303) as *B. duttonii* Novy and Knapp 1906, the agent of East African relapsing fever or African tick fever; *B. hispanica* de Buen 1926, the cause of Hispano-African relapsing fever; *B. duttonii* var. *crocidurae* Leger 1917 (synonymous with *B. crocidurae, B. merionesi,* and *B. microti*) and *B. dipodilli* Heisch 1950 as members of the "crocidurae" subgroup; *B. persica* Dschunkowsky 1913 as the cause of Asiatic relapsing fever; *B. turicatae* Brumpt 1933, *B. parkerii* Davis 1942, and *B. hermsii* Davis 1942, the agents of

the disease in the United States; *B. venezolensis* Brumpt 1921, the cause of South American infections. This classification takes into account disease, geographical distribution, natural vectors, and animal reservoirs (if known). The names of the borreliae are closely linked with those of their vectors, principally because agent-vector specificity is very strong in borreliae (118, 223, 386). *B. recurrentis* is propagated by the body louse *Pediculus humanus* Linnaeus 1758, the other species by *Ornithodoros* ticks. These relationships will be discussed in detail later.

Davis (219, 220) called attention to difficulties in studying the specificity of strain-vector-host relationships. Some experimenters study the organism by injecting borreliae or crushed insects; others feed the vectors on infected animals. It is quite possible that different technics give divergent results. After extensive studies Baltazard (50) agreed with the concept of tick-specificity of borreliae, principally in the United States. The recent investigation of 2623 *O. canestrini* by Skrynnik (650) also tends to confirm this concept. We assume, therefore, that *B. duttonii* is harbored by *Ornithodoros moubata, B. hispanica* by *O. erraticus erraticus,* the "crocidurae" subgroup by *O. erraticus sonrai, B. persica* by *O. tholozani (=O. papillipes), B. turicatae* by *O. turicata, B. parkerii* by *O. parkeri, B. hermsii* by *O. hermsi, B. venezolensis* by *O. rudis (=O. venezuelensis),* while the vector relationship of *B. mazzottii* is not yet settled.

In laboratory experiments it may happen that a tick is used in transmission studies and that such a tick is already carrying a different strain of the same *Borrelia* or another *Borrelia.* Studies with wild ticks, therefore, always have to be evaluated carefully. In well controlled experiments, however, some unusual *Borrelia*-tick relationships have been observed. For instance Brumpt (117) found transmission of *B. crocidurae* by *O. moubata* and *O. marocanus.* Davis and Burgdorfer (226) isolated a strain of *B. parkerii* in Oregon which was transmitted not only by *O. parkeri* but also by *O. turicata.* Davies (222) listed further exceptions. Brumpt (122), a firm believer in the unitarian acarine concept, invited attention to possible mutations of the borreliae in the host that may influence the outcome of such studies. Walters (709) and Felsenfeld (264) believed that *Borrelia*-tick species specificity follows the

rules closely but is not always exact. Walton (713) went one step further by investigating the feeding habits of ticks and reached the conclusion that many acarines have a strong preference for a single mammalian host.

Nicolle and Anderson (521) believed that borreliae were originally parasites of small rodents and were later transferred by ticks to man and from man to lice. Thus, ticks conserve and lice propagate these organisms. They are systemic parasites of argasid ticks and lice according to Burgdorfer (129). Baker and Wharton (41) favored the concept that *Borrelia* developed with acarinae, primarily as a parasite of these ticks, and evolved into different strains with the genetic changes that differentiated the various *Ornithodoros* species. Mammals are merely accidental hosts of borreliae according to this concept—this seems to be accepted by most writers.

Morphology

According to the majority of observers (132, 303, 363, 364, and others), borreliae are helical organisms, 3 to 25 μ long, usually 10 to 20, and 0.2 to 0.5 μ wide according to their environment and the time that has elapsed since division. Forms as short as 8 μ and as long as 40 μ have been described. They have 4 to 30 coils which are uneven, especially when the smears have been dried. Manson and Thornton (467) in Indochina and Sibilia (646) in Ethiopia reported short as well as long forms, whereas Hindle (364) described slender organisms in Central Africa. Passage through animals may result in the appearance of thicker variants (69). Aristowsky and Hoeltzer (24) observed irregular, bizarre spirals. Such forms and fragmented, conglomerating borreliae are not rare in the blood of relapsing fever patients just before the crisis. They vary in appearance (and antigenicity) during subsequent attacks in the same person (669), but variations are even more frequent when an unnatural mammalian host is inoculated with them (687). In dark field microscopy, Baltazard (47) saw a luminous contour around some strains instead of the usually uniform refraction.

Flagella were described in a so-called *B. novyi* strain maintained in the laboratory by rat passages (441). This finding was

not confirmed by electron microscopy. The structure of *B. novyi* was studied by Lofgren and Soule (456), who saw a terminal filament and fragile fibers, as well as fixed and free granules in the protoplasm. Further electron microscopic studies were carried out by several other investigators (34, 35, 38, 109, 401, 402, 403, 498, 673). A foamy envelope that could be washed off with sodium deoxycholate, a cell wall formed by 2 membranes with 20 to 25 fibrils on its surface, no mitochondria, but no limiting membrane between cytoplasm and nuclear zone were seen. The cytoplasm became invaginated before cell division that took place by transverse fission. An activator membrane was also described that, according to Mölbert (498), is connected with the band of fibrils.

Pillot *et al.* (574, 575, 576) undertook a systematic study of *Treponema, Borrelia,* and *Leptospira.* All Spirochetaceae appeared to have an elastic envelope containing lipids, polyosides, and pro-

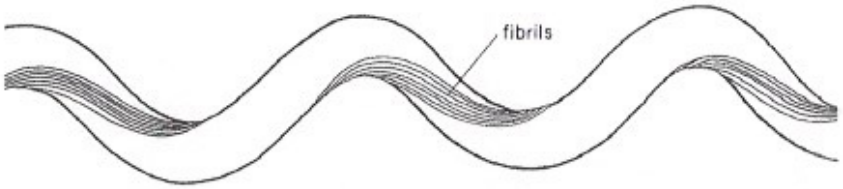

FIGURE 2. Structure of *Borrelia,* longitudinal view. Schematic drawing.

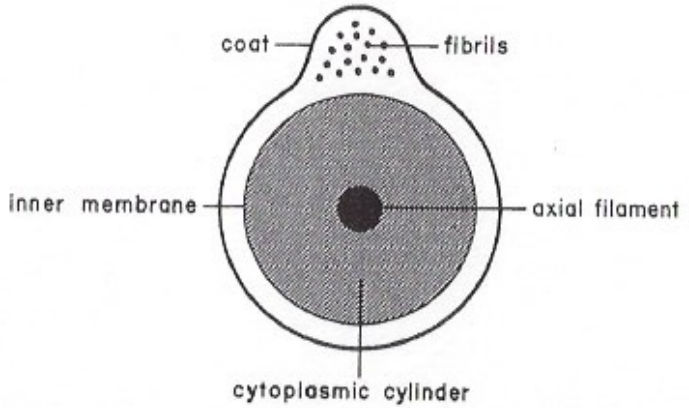

FIGURE 3. Structure of *Borrelia.* Cross section. Schematic drawing.

teins. Specific antigen is carried by the polyoside-lipid complex. The limiting cell body (parieto-cytoplasmic) membrane gives the organisms their helical shape and confines the protoplasm. It contains glucosamine peptides which confer solidity to it. The locomotory apparatus is situated between the envelope and the cytoplasmic membrane, consisting of parallel fibrils, which are coiled around the cell body. The fibrils and the cell body rotate at the same rate but in opposite directions. Intracytoplasmic mitochondria were not observed but mesosome-like structures were present. A long fibrillar nucleus without a limiting membrane was also seen. Aeschlimann *et al.* (10) studied blood forms of *B. duttonii*, *B. tillae*, *B. crocidurae*, and *B. hispanica*, as well as *B. duttonii* in *O. moubata*, with the aid of the electron microscope. An outer coat enclosing the central cytoplasmic core, with a lateral ridge running along the entire body of the organisms and harboring 15 to 22 fibrils, a cytoplasm with its own membrane and ribosomes, a central nuclear substance without membrane but running through the entire body and containing DNA were found in all examined species. *B. tillae* had an additional membrane, probably part of a mesosome. There was some difference in the degree of wrinkling of the outer membrane but no additional characteristics that would permit strain differential diagnostic characterising of the genus *Borrelia* were found. However, *Leptospira* having only one fibril, *Treponema* 3 to 7, and *Cristispira* with several hundred fibrils were encountered. Geigy (303) pointed out that the fibrils attached to granules may be similar to bacterial flagella. During cell division, each fibril has to split separately.

Electron microscopic studies put an end to the "granule" theory, which was promulgated by Leishman in 1907 (442) and Hindle in 1911 (361). It was believed that either "invisible," "filtrable," or "granular" forms represented a metacyclic development of borreliae because these organisms disappear from the blood between attacks, as well as from the gut of the vectors before they are found in the celomic fluid. Todd (685) and Leishman (442) reviewed the debate on granules; Baltazard and Habibi (58) on invisible or filtrable forms; Baltazard *et al.* (51), and Weyer (724) on metacyclic changes in general. Chorine and Crogue (169) called attention to the fact that short and small forms and presence of

organisms too few to be noted may be the reason for misinterpreted morphologic intravital changes in size and numbers. Heisch *et al.* (349, 353, 355) considered granules appearing in lice to be breakdown products. Burgdorfer (129) took a firm stand against the metacyclic theory while he observed a reduction in size of *B. duttonii* during its passage in the tick. Westphal (723), using the phase microscope, saw internal segmentation of borreliae that may give rise to granule-like fractions. Felsenfeld (264) studied granules which appeared as breakdown products and involution forms rather than stages of a metacyclic process in *B. turicatae*, under the fluorescent microscope. It is not believed therefore that borreliae undergo an evolutionary life cycle in the vector or in the host.

Hindle (363, 364) described the motion of these flexible organisms that are endowed with anterioposterior polarity (243) as cork-screw like, in forward and backward waves, and laterally by bending and looping. Geigy (303) emphasized the corkscrew-like motion as permitting the penetration of the organisms through mucous membranes and even through the skin. In the body, borreliae live in extracellular and interstitial fluid but may penetrate into cells. However, they do not enter red blood cells. Under the conventional light microscope, locomotion that may be suddenly reversed, and helical rotation and twisting, are usually seen. Ackermann and Protasov (1) studied the ring-like twisting of borreliae frequently seen during the last hours of the relapse and concluded that it is a protective measure for these organisms.

Multiplication takes place by transverse fission. Unusually long forms are due to lack of separation of the daughter cells. Details are described under electron microscopic observations above.

Biochemistry

Biochemical characteristics of borreliae as determined by electron microscopic methods were described in the preceding chapter. Studies by other methods are hampered by difficulties in culturing *Borrelia*, as pointed out by Geiman (314). Nevertheless, Fenyvessy and Scheff (272) found that dextrose is utilized by glycolysis. Cell-free extracts and homogenates of *B. recurrentis* follow the Embden-Meyerhof pathway (385). Oxygen is not utilized. The so-called *B. novyi* also utilizes dextrose but accumulates excess lactic acid

(626). Scheff and Kutner (627) studied the dehydrogenase activities of *B. recurrentis*. Smith (653, 654) demonstrated in *B. recurrentis* homogenates hexokinase, P-glucoisomerase, P-fructokinase, aldolase, P-glyceraldehyde dehydrogenase, triose P-glycerate kinase, P-glyceromutase, enolase, pyruvate kinase, and DPN-dependent lactic dehydrogenase.

Both live and killed borreliae moved toward the cathode in the electric field (273).

Ginger (318) attacked the problem of classification of borreliae by biochemical means. Van Thiel (682) considered them protozoa; Lewin Cyanophyceae. Ginger chose the study of cell wall mucopeptides as a pivotal point. These yield, on hydrolysis, amino acids and sugar together with amino sugar glucosamine, galactosamine, and muramic acid. Muramic acid is present in true bacteria, Actinomycetales, Cyanophyceae, and Rickettsiae. Muramic acid was present in the *B. duttonii* strain tested by Ginger. It was also susceptible to those antibiotics that inhibit bacteria and was sensitive to lysozyme, which is a characteristic of bacteria. Moreover, there was a cell wall, and resistance to nuclear staining methods, which are not found in protozoa.

Felsenfeld *et al.* (265) studied the biochemical and physical properties of the antigenic fractions of *B. parkerii*. Two protide fractions were relapse-specific, the third common to borreliae.

It is evident that the problem of biochemical composition and metabolism of *Borrelia* requires much further study.

Immunology and Serology

Classifying *Borrelia* serologically is a very difficult task. Jancsó (383) compared the characteristics of strains collected from different relapses in the same patient, and found that cross-immunologic phenomena are not the rule. This led to recognition of the fact that borreliae undergo phase variation and develop antigenic variations during subsequent attacks by the same organism in man and animals. Beck (70) saw similar results using the protection test by inoculating animals previously infected with homologous strains collected from subsequent attacks. Several investigators (264, 303, 364, 365, 619, 630, 667 and others) reviewed this problem. Russell (618) attempted to establish "serotypes" (phases)

and designated them by the use of capital letters. Experimenting with large West African rats *(Cricetomys gambianus)*, she found that only serotypes A and B conferred life-long immunity. In later investigations Russell (619) found that the serotype of the variant strain recovered from a later relapse in the rat that has been inoculated with the variant will be serologically identical to the organism isolated from the patient during the attack that followed the relapse from which the *Borrelia* was originally isolated. She concluded that *Borrelia* can adapt itself repeatedly to the antibodies of the animal although the number of re-adaptations is limited.

Ashbel (28) studied 17 strains of *B. persica* in 110 guinea pigs which are strongly susceptible to this *Borrelia* strain. He found that immunologic variants occur more often in man than in animals. Serologic variants have been observed also in monkeys, and in one tick vector. The original *Borrelia* strain did not protect against infection with relapse strains in some instances. However, relapses occurred with and without the development of variants.

Schuhardt and Wilkerson (632) infected rats with single organisms of *B. turicatae* and found that serologically different variants emerged. The immobilizines and lysins that developed against them were different, however. In man, Sterling-Okunewski (669) noted that serologic variants appeared in consecutive attacks regardless of the number of circulating borreliae. Ackermann and Protasov (1) considered it possible that the immunity conferred by the first attack affords only relative protection. Schuhardt (630, 631) defined the relapse phenomenon as the result of the inherent capacity of borreliae to undergo one or more antigenic variations. Cunningham (211) stated that there is a tendency to revert to a previous variant phase during subsequent relapses and that the dominant phase may be stable or not in various strains.

Cunningham *et al.* (213) experimented on squirrels *(Sciurus palmaris)* and monkeys. They described 9 phases and labeled them according to their sequence in consecutive attacks, from A to I. Phase A from the first attack, B from the first relapse, and D and E from the second relapse were complementary to each other. Phase C from a second attack in a mixed infection, and D and E from the second relapse were related to B; phase G from a second attack

was related to phase A. Phase F was rare, whereas, H and I developed in prolonged relapses, when phase B was at a low ebb. In man, mostly A and B were observed, with C, F, and G occurring less often, and with a tendency to revert to A or B. This study demonstrated the intricacies of the serologic structure as well as the coexistence of several variants. The practical application of the finding of more stable phases was tested by Cunningham and Fraser (212) on the Northwest Frontier of India. Most sera in that area did not react with phase B of the louse-borne *Borrelia*. Type C gave positive reactions.

Tick-borne borreliae appear to undergo more variations than louse-borne species. Coffey and Eveland (185) described 4 subsequently developing serotypes of *B. hermsii*. They designated them O - A - B - C. A tendency to revert to phase O was noted. Variants were also found in ticks by Cunningham and Fraser (212). Some investigators do not agree with these authors (28). Neither the antigenic schedules of Cunningham nor the six phases of *B. recurrentis* of Meleney (490) who studied *B. recurrentis* in splenectomized squirrels *(Sciurotamias davidianus)* and chipmunks *(Eutamias asiaticus)* attained popularity, even though the latter emphasized only phases A and B which he found reversible. Considering that Toyoda (688) observed phase variations also depending on the treatment of the patients, the great number of strains, substrains, and mutants, all of them producing a number of different variants under varying conditions delineated by their own microclimate, one has to agree that extreme caution is necessary in making generalizations from limited studies. Unfortunately, type collections of borreliae do not exist to the knowledge of this author. This hinders even further comparative and comprehensive studies of this aspect of borreliosis.

Serologic and other studies of borreliae are further impeded by the occurrence of partly related, partly dissimilar strains in the same locality or in nearby localities. Some instances have been mentioned previously. Dubois (247) observed such strains within the radius of 80 kilometers in the Congoes. Geigy and Burgdorfer (303, 307) had a similar experience with *B. duttonii* strain labeled B. Infection protected mice against strains C and D but not *vice versa*. They called this a one-sided immunity. Addamiano and Babudieri (2) observed the same phenomenon with two strains designated Irbid and Husu, respectively, in Jordan, and coined the term "asymmetric immunity." Cross-protection between different species, at least to a moderate degree, was described among members of the crocidurae subgroup (188), in bush-babies and not regularly in primates between *B. recurrentis* and *B. duttonii* (296), and not between *B. recurrentis, B. duttonii,* and the North American tick-borne species (193, 194). Thus cross-protection is not always the rule. Reciprocal immunity may or may not be present. This restricts the value of neutralization and cross-protection tests in animals.

Serologic methods feasible for routine laboratory work were reviewed by Schuhardt (630) and Wilson and Miles (735). They disagree on the value of the agglutination test. It appears that a feasible agglutination or precipitation test has yet to be developed because technical difficulties, principally the limited number of organisms available, the complexity of the test in Treponemataceae (363, 364), cross-reactions with *Treponema* (12, 623), and autoagglutination thwart efforts to demonstrate agglutinins with ease and certainty.

Brussin (125) studied the adhesin phenomenon. It was found feasible for pratical use when only few borreliae are present in the blood (630). Adhesins appear later during the disease (364). Adler and Ashbel (3) described a factor causing adhesion of borreliae to leukocytes and, if the protoplasm of these cells is destroyed, also to white blood cell nuclei. Adhesin is independent from lysin in *B. turicatae* and *B. parkerii* (265). Mooser (500) observed that *B. duttonii* show mutual adhesion, and display this phenomenon not only with leukocytes but also with red blood cells, and on the bare surface of slides and coverslips. He was able to prevent adhesin activity by homologous but not by heterologous serum.

Borrelia enters leukocytes even in the absence of phagocytic activity, by pinocytosis. While Adler and Ashbel (3) did not observe phagocytosis, Belezki and Umanskaia (72) recorded some such activity by elements of the reticuloendothelial system (RES), including monocytes and histiocytes. In the central nervous system, glia cells and Hortega cells acted as phagocytes. Similar relationships exist in mice, after RES blockade and splenectomy (126).