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BORRELIA: STRAINS, VECTORS, HUMAN
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Oscar Felsenfeld, MSc, MD

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Warren Harold Green
1915-1992

GREEN, WARREN HAROLD, publisher; born Auburn, IL, July 25, 1915; s. John Anderson Logan and Clara Christina (Wortman) G.; married Joyce Reinerd, October 8, 1960; deceased June 6, 1992. Student, Presbyterian Theological Seminary, 1933-34, IL Wesleyan University, 1934-36; M.B., Southwestern Conservatory, Dallas, 1938; M.M. St. Louis Conservatory, 1940, PhD, 1942; H.L.D. (hon.) Southeastern University, New Orleans; L.L.D. (hon.) Institut de Droit Practique, Limoges, France, 1983; D.D. (hon.), California Theological Seminary, 1980; Litt.D. (hon.), Confederation Europeene de L'Ordre Judiciaire, France, 1983. Professor voice, composition, conducting and aural theory at St. Louis Conservatory, 1938-44; program director U.S.O., 1944-46; community service specialist Rotary International, Chicago, 1946-47; editor in chief Charles C. Thomas, Pub., Springfield, IL, 1947-66; publisher, president Warren H. Green, Inc., St. Louis, 1966-1992, Warren H. Green International, Inc., 1970-1992; managing director Publisher's Service Center, St. Louis, Chicago, and Longview, TX, 1967-92; vice president Epoch Press, St. Louis, 1986-92; cons. U.S., European publisher's professional societies; lecturer: medical publishing, Civil War. Contributed articles to professional journals, books on Civil War history, writing, editing. Recipient Presidential citation for outstanding contribution to export expansion program U.S., 1973, citation MD Crime Investigating Committee, 1962, citation International Preventive Medicine Foundation, 1977, citation AMA, 1978. Member Mayor's Committee on: Water Safety, Metropolitan St. Louis Art Museum, MO Botanical Gardens. Member: Civil War Round Table (v.p. 1969-1991), American Academy Criminology, American Academy Political and Social Science, American Association Medical Book Publishers, American Judicature Society, Great Plains Historical Society, St. Louis Philharmonic Society, MO Botanical Gardens, St. Louis Art Museum. Clubs: MO Athletic, Media, Elks, World Trade, Direct Marketing St. Louis.

"As God gives us the ability to seek the truth, know the truth, and be true to others as well as ourselves, and as God gives us the understanding to maintain a high sense of values in our work and in our relations with all fellow men, it naturally follows that attitudes, endeavor, love of people and all other living things, cause one to like and be liked, which is the first step towards any worthwhile goal."

BORRELIA
Strains, Vectors, Human and Animal Borreliosis

Oscar Felsenfeld, MSc, MD

PREFACE

Despite periodic reports that tick-borne relapsing fever occurs sporadically over more than half the area of the entire Continental United States, the numerous deaths due to this disease in the drier parts of Asia and Africa including the thousands who become its victims in Ethiopia and the Sudan every year, the unknown toll in lives and suffering that the disease is still taking in Central and South America, and the ever-present danger of recurrence of epidemics should the louse-borne form burst out of its present confines if or when a disaster creates favorable conditions for such spread, only scanty notes on relapsing fever can be found in the medical literature of the United States today.

One is amazed at the confused nomenclature and the mass of misinformation concerning the transmission and vectors (some of which has managed to creep into even respectable textbooks). The considerable variations in the clinical picture during various outbreaks and in widely separated localities, and the unusual immunologic conditions that accompany the infection should be emphasized so that the medical profession can be alerted should an outbreak occur in this country.

The genus Borrelia *that causes human relapsing fever includes some species that are of interest to the dentist (*Borrelia vincentii*), and to the veterinarian (*B anserina, B theileri*).* Borrelia *infections appear in man as relapsing fever and in animals as so-called fowl spirochetosis, or tick-transmitted spirochetosis in cattle, which are clinically defined entities. This monograph deals principally with human relapsing fever and fowl spirochetosis, while exploring other conditions in which borreliae may play a role. The discussion of the borreliae and their vectors, the pathology caused by them, and other features will be presented in separate chapters discussing borreliosis as human relapsing fever, cattle and fowl disease, and Vincent's angina, respectively. Each of these chapters will deal separately with the species of Borrelia involved, and with the consequences of the infection. Borreliae that have been isolated from vectors only, and not from man or animals, will also be enumerated.*

CHAPTER 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 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,[363-365] 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.

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 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 USSR, 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,
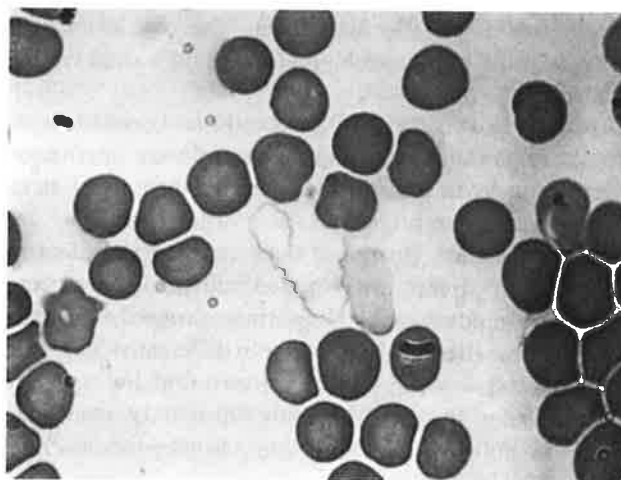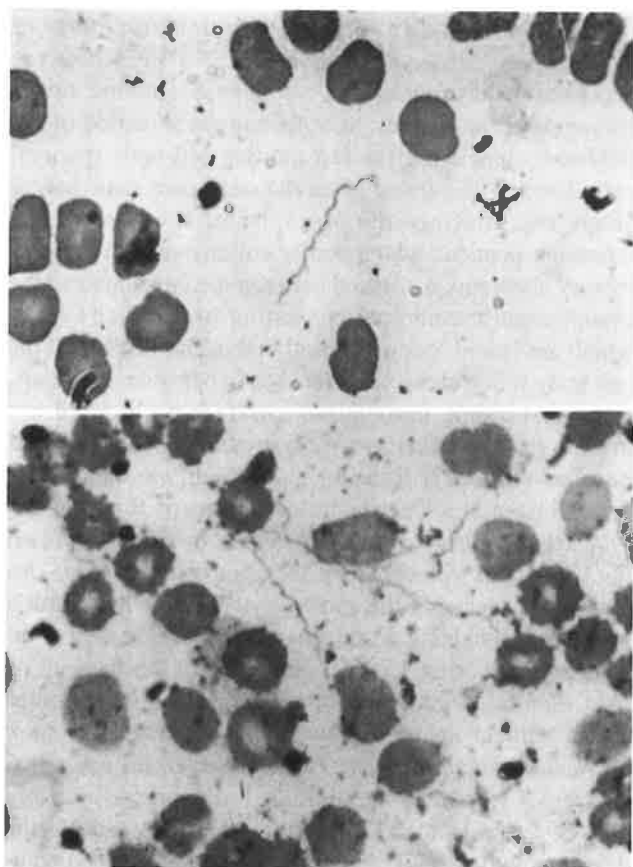
Figure 1. Borrelia in blood. Giemsa stain ×950.

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 substrains 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 techniques 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 0.02 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[574-576] undertook a systematic study of *Treponema, Borrelia,* and *Leptospira.* All Spirochetaceae appeared to have an elastic envelope containing lipids, polyosides, and proteins. Specific antigen is carried by the polyoside-lipid complex. The limiting cell body (parietocytoplasmic) 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 corkscrew-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 lifelong 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 readaptations 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

Table 1. Borreliae causing human relapsing fever, their vectors, and geographical distribution.

Borrelia	Vector	Geographical Distribution
B recurrentis	Pediculus humamus	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		Last 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 America, Texas
B turicatae	O turicata	Central and South America, Western US, Canada
R parkerii	O parkeri	Western US, Canada
B hermsii	O hermsi	Western US

*Human pathogenicity low.

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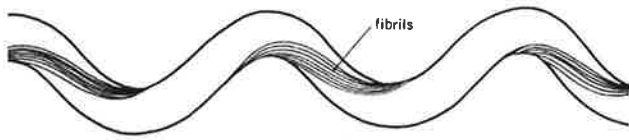
Figure 2. Structure of Borrelia, *longitudinal view. Schematic drawing.*

Figure 3. Stucture of Borrelia. *Cross section. Schematic drawing.*

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 practical 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 homol-

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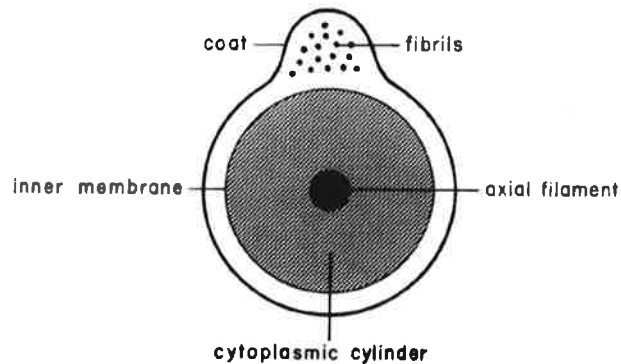
Borrelia enters leukocytes even in the absence of phagocytic activity by pinocytosis. While Adler and Ashbel[3] did not observe phagocytosis, Belezki and Umanskaia[72] recorded some such activity by elements of the reticuloendothelial system (RES), including monocytes and histiocytes. In the central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

Immobilizines were said to be of small molecular size[449] and are related to β and γ globulins.[136] They were observed also in the heavy (19S) IgM.[268] Immobilizines can be found in the serum long after the infection has subsided[268,449,450] and may be directed against a specific phase of the causative agent.[598] These antibodies are present also during the latent phases of the disease. They are strain specific.[697] Levaditi et al[450] expressed the opinion that antibodies such as immobilizines may induce phase variation in borreliae.

The borreliolytic activity of the serum of infected man and animals appears to be identical to antiborrelial cytolysin and borreliocidin.[262] It was considered inconstant in guinea pigs infected with *B hispanica* but in man it persisted for nearly a year.[572] Toyoda[688] had the same experience. Ranque et al[597,599] found it highly specific, whereas complement fixation test, fluorescent microscopy, and skin tests showed cross-reactions. The cardiolipid hapten and group proteins are, however, common to related organisms.

Toyoda[688] and Wolstenholme and Gear[737] described complement fixing antibodies in relapsing fever which will be further discussed in the chapter on Laboratory Diagnosis.

Immunity to borreliae has been termed a premunition-like phenomenon by Geigy and Burgdorfer.[307] The studies of Chamsa[153] led him to the conclusion that this premunition is strain specific.

Loewy[455] correlated the periodic changes of body temperature during relapses ending with crisis, and the lesions often observed in the nervous system in the course of the disease, principally when treatment has been started late. Therefore, he concluded that anaphylactic phenomena play a role in relapsing fever.

Little has been said about natural antibodies in borreliosis. Weichbrodt[719] questioned whether they may not be present in the cerebrospinal fluid. Such antibodies, or the lack of some physiologic capabilities of certain *Borrelia* strains, could explain why not all borreliae invade the central nervous system.

Staining

Borreliae have an affinity for acid dyes, whereas many other bacteria prefer basic dyes.[720] Nevertheless, borreliae can be stained with practically any aniline dye.[132] Azureosine and related stains of Leishman, Giemsa, May-Grünwald, Romanowski, Wright, and their combinations are favored for staining blood films from patients and animals.

Du[246] described a simple and effective method feasible also for the staining of thick blood smears. The slides are dehemoglobinized with 6% acetic acid in 95% ethanol, rinsed, and then stained with carbolfuchsin for one minute.

Pampana[561] stained thick drops with a 2% methylene blue B extra solution in distilled water, to which 4 ml formol and 10 ml glacial acetic acid were added after filtration. Methylene blue was used also by Simons[648] who mixed 1 ml saturated methylene blue solution in physiologic saline with 2 ml 10% sodium taurocholate in saline, added 2 to 4 loopfuls of this mixture to an equal volume of blood, then made smears with it on microscope slides. This method can be used also for the examination of citrated blood which first has to be centrifuged and then the sediment can be examined.

As most workers do who use routine blood stains, Coles[196] also recommended prolonged staining but employed orange tannin for differentiation after the coloration. Vago[696] and Young[743] recommended mercurochrome. The latter employed concentrated aqueous mercurochrome for 3 minutes, followed by concentrated aqueous methyl violet. Our group[264] applied 1% crystal violet for a few seconds after staining according to Wright. Other combinations, such as the use of saturated alcoholic or aqueous solutions of a basic dye followed by an acid dye in 30% alcohol (gentian violet and acid green, or brilliant green and acid fuchsin) were recommended by Weiss.[720] Levine[451] used careful fixation of airdried smears, first with acid-free chloroform, then with acid-free absolute ethanol. Fuchsin was recommended for staining.

Fluorescent antibody studies of borreliae were made by Coffey and Eveland[184] who found them superior to the immobilizine and lysin tests. Maestrone[463] used the fluorescent antibody method for leptospiral and viral antigens in formol-fixed tissues that is applicable also to borreliae. The tissues are refixed with acetone for 5 minutes, dried at 37°C, exposed to ammonia vapors for 2 days at 37°C, or acted upon with 1% ammonia for 3 to 5 minutes. Sodium bisulfite, 25% for 5 minutes, may be substituted. The slides are washed with 3% Tween 80 containing saline buffer pH 7.2, blotted dry, reacted with rabbit anti-*Borrelia* serum or globulin, then, after washing with buffered saline, sandwiched with a fluorescein isocyanate or rhodamine labeled anti-rabbit serum, and mounted in glycerol. A slight shrinkage is usually apparent.

Silver impregnation methods are used for the visualization of borreliae in tissues. That of Krajian gives excellent results and will be described in the Appendix in detail.

The methods of choice will be discussed in the chapter Laboratory Diagnosis.

Culture Methods
In Vitro

Noguchi[544,545] succeeded in growing *B recurrentis* and *B duttonii* in a rabbit kidney-ascitic fluid medium, under liquid paraffin seal. Maximal growth was observed in 7 to 9 days. First, short, then longitudinally dividing forms were seen. Noguchi succeeded in passing the Koch strain 29 times over a period of about 6 months in this medium but others[262,513] were not successful in attempts to culture borreliae from the blood and organs of patients using this medium. Others[658] had better results with laboratory strains. Kligler and Robertson[414] pointed out that the medium should be slightly alkaline. These authors used ascitic fluid, horse or rabbit serum, 1% peptone broth, or egg albumin solution. Moroder[504] employed a mixture of inactivated rabbit or horse serum with 2 to 5 parts of physiologic saline, and covered the cultures with liquid paraffin. Granules still present in old cultures were infective for mice. Li[452] dispersed the yolk of one egg in 400 ml physiologic saline and added egg white. After coagulation, liquid paraffin was layered over the slants. One or 2 drops of citrated blood were put into the supernate when transfers were made. Chorine and Crogue[169] also used blood. Their medium contained peptone water, fresh rabbit serum, Tyrode's solution (which could be omitted), and laked or defibrinated human blood. It took 7 to 8 passages to establish the slowly growing strains. Others[44] were not successful with the medium of Chorine and Crogue.

Wolman and Wolman[736] prepared their medium by adding 10 ml human ascitic fluid to 1 ml coagulated egg albumen. An equal volume of buffer pH 7.8 and 2 volumes of 1% dextrose were added. After covering with liquid paraffin, the mixture was held at 56°C for 1 hour each

on 3 consecutive days. *B recurrentis* lived and multiplied in this medium for 8 months but lost its virulence after a year. Krylova[426] had good results with a modified Wolman procedure.

It should be noted that most authors who succeeded in growing borreliae *in vitro* did so at 28°C to 30°C, or at even lower temperatures.

There is little hope that any of these methods could be used successfully in routine diagnostic laboratories. Further investigations may lead to the development of more feasible methods.

In Developing Chick Embryos

Chabaud[150] inoculated *B recurrentis* and *B duttonii* containing defibrinated and centrifuged blood on the chorioallantoic membrane (CAM). The organisms multiplied after 3 to 5 days but the embryos died on the 6th to 7th day. The incubation period was reduced after repeated passages. Experiments with defibrinated blood were more successful than with citrated blood. Oag[549] reported in the same year that *B duttonii* does not cause the death of developing chick embryos. The motility but not the virulence of the organisms increased after serial transfers. Later[550] Oag found that when chick embryos were inoculated 2 to 3 days before hatching, the borreliae were detectable in the blood of the chickens for about 5 days. Blood or serum from mice and fowl and of the chick embryo was borreliocidal *in vitro* but not *in vivo*. Oag could not offer an explanation for this phenomenon.

Other authors[91,158,334] were satisfied with the feasibility of chick embryos to support the propagation of borreliae, principally when inoculating 7 to 12 day old fertilized eggs. More than 35 passages were possible in 4 months.[334] The use of fertilized chick embryos, inoculating them either just under the CAM or into the yolk sac, has become an important diagnostic aid because relatively few animals are susceptible to *B recurrentis*. Chen[158] observed growth on the 5th day but the borreliae also died when the eggs expired. Rodhain and van den Bergh[609] stated that borreliae that attack adult fowl do not grow in developing chick embryos but that *B duttonii* gave good results in 10 day old embryos. Several investigators[40,44,76] were satisfied with this method but found the transferability somewhat irregular.

Tissue Cultures

Manteufel and Dressier[468] prepared tissue cultures from allantoic membranes and found that *B hispanica* multiplied on them. Our group has been studying the adaptability of *B turicatae* to several cell lines but without much success.

Maintenance at Low Temperature

Sparrow[665] called attention to the numerous factors that influence the survival and the virulence of borreliae, particularly of *B recurrentis*. Geigy and Sarasin[310] discussed the control exerted by the environment over *B duttonii*. While Hindle[364] stated that borreliae survived only about one day in sealed slide preparations, they could be kept alive in citrated blood for 3 months at 0°C to 2°C but were killed in 30 minutes at 50°C. Ackermann and Protasov[1] found that borreliae retained their virulence in the refrigerator for more than 100 days but Hindle[364] observed a gradual diminution in the number that remained virulent. Beck[69] found that North American borreliae died in frozen animal tissues after a few days but survived in sheep blood for more than 6 months. Bourgain[105,106] kept *B persica* alive at 4°C for 19 days, at 11°C to 15°C for 7, and at 37°C for 4 days. They died in isolated mammalian organs in the refrigerator in 7 days but in cadavers at room temperatures in 4 days. Temperature between -15°C and -20°C killed them in 2 days, whereas others[726] kept borreliae alive at -72°C for several years. Kemp et al[405] found, however, that *B turicatae* is killed by sodium citrate used to keep the blood from coagulating, and by freezing. Beck[69] also preferred refrigeration to freezing. Hanson and Cannefax[336] recommended lyophilization as a means of preserving borreliae. Lofgren and Soule[456] destroyed borreliae by repeated freezing and thawing.

Borrel and Marchoux[103] found that borreliae multiply best in ticks at 35°C. Leishman[442] also stated that borreliae degenerate in ticks more rapidly at lower temperatures. These observations are of importance for the understanding of the seasonal fluctuations of tick-borne relapsing fever.

These and similar findings, coupled with the tediousness of the attempts at culturing borreliae in test tubes or in developing chick embryos, led to experiments directed at maintaining them in their vectors and hosts.

Maintenance in Vectors and Host Organs

The longevity of some ticks carrying borreliae is remarkable.

Pavlovskii and Skrynnik[569] kept *O tholozani* alive for 16 years at 15°C to 18°C. They had to be fed only once a year. Larval stages could starve 15 months; nymphs 2 to 11 years; and adults 10 years. Pavlovskii and Skrynnik[568] also observed that *O tholozani* could starve 7 1/2 years and remain alive, transmit *B persica* after 12 years, and live for 25 years. Mooser[501] found *O moubata* alive and carrying its *Borrelia* for 2 years. Brumpt[120] stated that borreliae can be preserved in their vectors at 5°C to 7°C for several weeks, without loss of virulence.

It has become common laboratory practice to keep ticks infected with borreliae in a sandbox, or in test tubes with a strip of filter paper running along the center of the

FROM FIRST (I) AND SECOND (II) BORRELEMIA
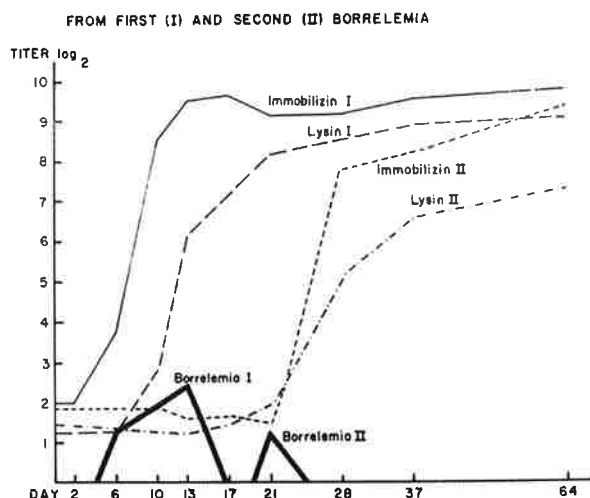
Figure 4. Serologic data, patas monkey (Erythrocebus patas) *infected with* Borrelia parkerii.

tubes. The ticks are fed on young, preferably newborn mice. If dead animals are offered for food (the *Ornithodoros* ticks live only on blood), the cadaver may be put into the sandbox ("tickorium"). Not all individuals in the colony will be infected or transmit borreliae to their progeny. The maintenance of proper humidity may cause difficulties. Nevertheless, the long survival of borreliae in their vectors is an excellent means of keeping them alive, handy, and ready in the laboratory.

Another method of maintaining borreliae in the laboratory is preservation in the brains of infected rodents. Ashbel[28] was able to preserve *B persica* in guinea pig brains for 380 days. Toda and Hiroki[683] failed in their attempts at preserving the Manchurian strain of *B recurrentis* in mouse brain. Delpy and Rafyi[235] stated that guinea pigs are not susceptible to louse-borne borreliae but only to the tick-borne strains. They were able to maintain *B persica* in mammalian brain for 8 to 73 days. Sergent[640] transmitted *B hispanica* and found it in a guinea pig brain 3 years after it had disappeared from the blood. Mathis and Durieux[479] made passages of *B duttonii* in animals every 3 to 4 weeks. Fatal infection did not ensue, but inoculation of infective blood did kill the recipient mice. Pirot and Bourgain[579] called attention to individual variations in the susceptibility of guinea pigs. Some of the guinea pigs lived more than 7 months, in others the cerebral infection disappeared in 45 days. Sergent and Poncet[643] experimented with *B hispanica* in rats for which it was less frequently fatal than for guinea pigs. The route of inoculation greatly influenced the outcome of the research, the subcutaneous inoculation causing appearance of fewer borreliae in the blood than intraperitoneal administration of the organisms.

Pampana[561] sacrificed guinea pigs 6 months after infection, using chloroform, washed the brains with saline, emulsified them, and then injected the emulsion into fresh animals. The incubation period was 6 to 12 days.

Weyer[725] studied different methods of preserving *B duttonii, B turicatae,* and *B crocidurae.* They remained alive when quick frozen at -76°C. *B recurrentis* in lice, and tick-borne in *Ornithodoros,* remained alive in the deep freeze for years. Even more effective was the propagation of *B recurrentis* by inoculation into the hemolymph of lice. When borreliae were numerous, they could be frozen in rat blood. Weyer's method can be recommended provided the arthropods are not thawed and refrozen.

BORRELIAE AND THEIR VECTORS

It will be seen on subsequent pages that it is difficult, perhaps even impossible, to speak about species of *Borrelia.* All of these so-called species may well be the variants of one single organism adapted to different environments programmed by vectors, hosts, and their mutual relationship. However, following the present custom of classifying borreliae according to their vectors (which is of considerable epidemiologic interest), the vectors will be discussed together with the "strains" they usually carry, or are said to harbor. Experiments with cross-infections of vectors will be listed, as well as strains that have been described but either were lost or were found to be mere variants of established *Borrelia* types. It is necessary to present separate discussions of the louse with *B recurrentis* and ticks with their borreliae for reasons which are evident.

The Human Louse and *Borrelia recurrentis*

As mentioned before, Mackie was the first to incriminate the human body louse as the vector of epidemic relapsing fever. Nicolle and his coworkers[531,532,537,538] worked out many details of the louse-*Borrelia* relationship. Nicolle and Anderson[521] believed that the contemporary strains of *B recurrentis* were derived from tick-borne strains. Adler and Ashbel[4] agreed with this concept.

Lice

General accounts of the life cycle of the louse and of the mode of transmission of borreliae by this insect have been given by numerous authors. Nicolle et al[534] found that the organisms are not transmitted to the progeny of lice. Chapcheff[155] and Chiao[163] emphasized that only *Pediculus humanus corporis (vestimenti)* and *Pediculus humanus capitis,* ie, the clothes or body louse, and the head louse, respectively, but not the pubic or crab louse, *Phthirus pubis,* transfer relapsing fever organisms. This

was confirmed by data in the monograph on lice by Buxton[135] and in the textbook of Horsfall.[374] Thus we are concerned only with the human body louse and the closely related head louse.

The genus *Pediculus* is a member of the family Pediculidae that belongs to the order Anoplura (Siphunculata) or sucking lice. The body louse, *P h corporis* is also called *P humanus humanus*. The head louse is about 2.5 mm long and slightly smaller than the body louse but they can interbreed. Lice are strictly host-specific. Geigy[303] stated that head lice must have preceded body lice and adjusted themselves to man before he started to wear clothing. Lice cling to hair. The body louse does not invade the head hair and beard but *P h capitis* may migrate to the body. *P h corporis* lives also in the folds of clothing, principally in the underwear. The fertilized female lays about 300 eggs which adhere to hair or clothing and hatch at 28°C to 32°C in 8 to 9 days. Three larval stages develop within 9 to 10 days. The larvae and nymphs descend to the skin and feed. One adult louse takes up about 1 mg blood at one meat but it is possible that smaller amounts are consumed and then the lice feed more often. Feeding is rapid and followed by a quick evacuation of feces. If the louse has a meal on a patient with *B recurrentis* in his blood, the organisms reach the stomach of the louse but many are destroyed. Heisch et al[355] found that the density of the borreliae in the blood consumed by the louse must be at least one or two per oil immersion field to make the meal infective.

The borreliae pass from the gut into the hemocele (celomic cavity) where they multiply. In the louse, organs like the salivary glands, the ovaries, or the Malpighian bodies are not invaded. This precludes hereditary transmission. Only about 12% of the lice fed on relapsing fever patients became infected in the experiments of Nicolle et al.[534] In one instance 4407 lice were fed on a patient, and none acquired borreliae. On the other hand, Riding and MacDowell[603] found that one half of the lice that were collected from persons who were ill with relapsing fever for 1 to 10 days were infected with borreliae. The organisms become visible in the celomic fluid (hemolymph) about 5 to 8 days after the blood meal. The borreliae remain in the louse until its death. Since borreliae are not present in the gut and salivary glands, they cannot be transmitted by the bite of the louse. Neither can they be propagated by fecal material. Borreliae may escape from the celomic cavity only when the louse is crushed.[179]

Heisch and Harvey[353] described several basic data on the relationship of lice and borreliae. They showed that the hemocytes of the louse may act as phagocytes and destroy some borreliae. These authors found borreliae also in the neural ganglion and nucleus but never in the salivary gland of the louse. The penetration of the borreliae into the hemocele took place from the anterior part of the midgut.

After a blood meal containing borreliae, the organisms disappear from the midgut of the louse within a few hours, to reappear in the hemocele after 5 to 8 days. This has been called the "negative phase." It has given rise to speculations about a filtrable phase of borreliae. The appearance of granules, short and corkscrew-like forms of borreliae, in the beginning of their sojourn in the celomic cavity has stimulated speculation and research also about metacyclic forms.[54,63,296,348,353,355] These investigations showed that a metacyclic development does not exist in the louse. The organisms become slender and small when penetrating the midgut but they can be found by diligent search. The "granula" theory is difficult to prove or disprove except with the aid of fluorescent microscopy, as our group did,[264] because "granules" occur naturally in the celomic cavity of lice.

The number of surviving borreliae is determined by the temperature and the hitherto not fully explored qualitative and quantitative changes of the gut juices of the louse. Wolman and Wolman[736] found for instance, that lice kept at 37°C were unable to infect man 1 to 18 days after a meal on a patient with relapsing fever.

The borreliae are tightly enclosed by the limiting membranes of the celomic cavity. Lice are delicate, however, and easily damaged. Their limbs and antennae are easily broken off. This permits the celomic fluid to flow out and to infect the site of the bite. This usually happens when a bitten person scratches himself. Scratching will also rub the borreliae into the skin.[179,537,538] Small children develop relapsing fever less often than adults. This may be because they seldom crush lice. In Europe, lice are crushed between thumbnails. In China and South America, lice are often popped between the teeth. A few authors[177] believe that putting lice into the mouth does not convey the infection but it has been shown[59,365] that borreliae may enter the human body through uninjured mucosae, including that of the gastrointestinal tract. The last practice may therefore lead to an increased number of relapsing fever infections.

After acquiring *B recurrentis*, the louse remains infectious for its entire life, which is about 3 weeks, sometimes longer.[63,134,453]

Experiments with louse-borne relapsing fever are hampered by the unwillingness of the human louse to bite other animals, except monkeys.[59,95,532] It has also been reported that it is possible to feed human lice on newborn rabbits and other newborn rodents.[208,209,219]

Human lice have a narrow temperature tolerance and die when it becomes too hot.[207] This has epidemiologic significance, which will be discussed later.

"Strains" of B recurrentis

Coleman[194] emphasized strain specificity but pointed out pitfalls encountered in cross-protection tests. Chen et al[160] used hamsters and monkeys to ascertain whether the Chinese and the American strains are identical with the aid of such tests.

Without the benefit of strain comparison, a *Borrelia* was isolated from a patient with relapsing fever at Bellevue Hospital in New York in 1907. This strain has been kept in numerous laboratories and used in animal and biochemical experiments. It is not known whether it was louse-borne or tick-borne. After literally thousands of rodent passages, this strain is widely used as a model for laboratory experimentation with borreliae under the designation *B novyi*. It is not certain whether conclusions reached from experiments with this "strain" are valid for other borreliae.

It was clear to Noguchi as early as 1912 that *B kochi* Novy 1907 was closely related to *B rossi* Nuttal 1908, and that both organisms, as well as *B carteri* Mackie 1907 and *B berbera* Sergent and Foley 1910, were local strains of *B recurrentis*. The author (OF) has been unable to find any laboratory that is still carrying either of these four strains. The description of *B aegyptica* is not clear enough to warrant its acceptance as a "species."

It is possible that some or all of these strains were tick-borne rather than louse-borne. Nicolle and Anderson,[520] working in Tunisia, believed that louse-borne borreliae can be transferred to ticks.

Baltazard and his coworkers[52] carried out extensive experiments by feeding lice and ticks on newborn rabbits artificially infected with borreliae. They found that lice could acquire infestation with *B microti, B turicatae,* and *B hermsii* by sucking blood of infant rabbits infected with these tick-borne strains. Numerous metacyclic forms appeared in lice infested by this method. Heisch and Garnham[349] fed batches of lice from a relapsing fever-free area (Nairobi) on monkeys infected with a *B duttonii* strain. The so-called negative phase (absence of visible forms in the insect) was shorter than in ticks; and the organisms appeared in metacyclic, corkscrew forms. This observation is important in the study of transmission of borreliosis because Heisch and Garnham found persons infested with lice living in huts in which *O moubata,* the carrier of *B duttonii,* was a common inhabitant. Heisch[341] believed, therefore, that the human louse can transmit *B duttonii* under natural conditions. Heisch[346,347] also noted a definite multiplication of *B duttonii* in the celomic cavity of lice 6 to 8 days after ingestion. The borreliae had a tendency to concentrate around the fat body in the head of the louse. Granular forms of the *Borrelia* also appeared. This may be a phenomenon related to life in an unusual vector, and perhaps it may also be a phenomenon of adap-

tation. Mooser and Weyer[502] could retransmit the borreliae to *O moubata. B duttonii* did not seem to be adversely affected during 21 louse passages. Boiron[97] succeeded in transmitting *B crocidurae, B duttonii,* and *B hispanica* to lice from infected mice. Weyer and Mooser[726] used rectal or intracelomic inoculation of lice, with *B duttonii, B turicatae,* and a crocidurae–group strain. Sparrow[660] confirmed that *B hispanica* can be adapted to the louse, and that small rodents may become reservoirs of louse-borne tick fever. Garnham[295] believed that lice may harbor *B hispanica* and that a man-louse-man cycle is possible, thereby forming a reservoir without passage through *O erraticus,* the tick-vector of *B hispanica,* and also bypassing rodents that are frequent hosts of this strain. Baltazard et al[54] experimented with *B crocidurae* and an antigenically distinct *B microti* strain. Lice were fed on patients, tritiated, and injected into human beings and animals. Of 62 individuals and rodents, 14 became infected. Talice,[675] however, did not observe infestation of lice with *B hispanica* when fed on infected man, mice, rats, and monkeys. Favorova et al[261] fed 4,658 lice on patients with tick-borne relapsing fever. The borreliae penetrated into the hemolymph in 1.25% of the lice but multiplied only in one. This group of investigators did not believe, therefore, that tick-borne borreliae can be transmitted to lice.

There appears, however, to be satisfactory evidence that tick-borne borreliae can be transmitted to lice. The antigenic stability of borreliae in insects is much greater than in animals. Probably repeated transmission cycles are required to establish variants and mutants with genetically modified characteristics that are relevant for such an adaptation. Baltazard[46] expressed this thought in considering *B recurrentis* a transient, inconstant form of *Borrelia* that has been modified by passages in rodents and ticks. We would add to this as a governing factor the man-louse biotope.

It should be mentioned that the monkey louse (*Pediculus longiceps*) is an excellent host of *B duttonii.*[342] In view of other extensive research on *O moubata (vide infra),* it would be perhaps somewhat rash to conclude that monkeys or *P longiceps* play an important role in the preservation of *B duttonii.*

Tick-borne Relapsing Fever
General Tick-Borrelia Relationships

Geigy[303] pointed out that ticks are arthropods but not insects. Ticks carrying the agent of human relapsing fever are classified in the phylum Arthropoda, class Arachnoidea (Arachnida), order Acarina, suborder Ixodides. The order Acarina includes also spiders and scorpions. Ticks are wingless; their body lacks segmentation into head, thorax, and abdomen; and a capitulum with mouth parts and palpae is on their ventral side. They have

Table 2. Size of some arthropods carrying Borreliae and their nicknames.[+]

Scientific Name	Male*	Female	Nickname
Pediculus humanus corporis	2.5-3.3 ×0.8-1.1	3.2-3.6 ×1.1-1.4	
Pediculus humanus capitis	1.6-2.1 ×0.6-0.8	2.4-2.8 ×0.9-1.1	
Ornithodoros moubata	4.2-5.8 ×3.7-4.2	7.8-12.8 ×6.9-10.2	Ochiopo, Tampan, Garrapato (Angola) Kufu, Bu (Zambezia) Kibu, Bibo (Uganda) Papasi (Zanzibar) Kimputu (Congoes) Curdud (Somaliland)
Ornithodoros erraticus	2.8-4.2 ×1.8-2.5	4.2-6.8 ×2.4-4.1	
Ornithodoros tholozani	3.5-6.2 ×2.8-5.2	7.6-9.1 6.8-7.4	
Ornithodoros rudis	3.3-4.2 ×2.4-3.3	4.8-6.4 ×2.8-4.2	Cuescas, Mordijini (Venezuela) Talajas (Colombia)
Ornithodoros talaje	4.8-6.2 ×3.4-5.2	5.3-7.4 ×4.7-6.2	
Ornithodoros turicata	2.7-4.4 ×2.3-2.9	4.6-6.9 ×3.3-4.3	Pajaroello (Mexico)
Argas persicus	4.0-5.5 ×2.6-3.3	5.0- 10.1 2.4-7.5	

*In mm, length × width.
Size often depends on time since last feeding.
[+]Nicknames according to Brumpt, E.: Précis de Parasitologie, 6th ed. Mason & Cie., Paris, 1963.

four pairs of legs (larvae only three pairs) which are articulated and equipped with terminal claws. They have many characteristics, however, of true insects, as the Malpighian (excretory) tubules, a tracheal breathing system, and a chitinous matrix of the hypodermis.

The suborder Ixodides consists of two main families: Ixodidae or hard ticks with 8 genera and about 400 species, and Argasidae or soft ticks, with 4 genera and about 120 species.[543] Argasidae have a softer and more elastic surface than Ixodidae. They have usually only one or two principal hosts, whereas Ixodidae are willing to feed on several different animal species. Argasidae attach themselves to the host for the blood meal for only a short time and take up less blood at one feeding but their body expands considerably during the feeding. Then the females, if fertilized, lay 100 or more eggs. The number of eggs is limited (some other ticks lay them by the thousands) and the female does not die after oviposition. This fertilization and egg laying is repeated several times during life. The genus *Ornithodoros* of the family Argasidae has no scutum; the margin of the body is thick, rounded, without definite sutural line (as Argas); and the hypostome has well-developed teeth, The integument is mammillated.[371,543] *Ornithodoros* (formerly spelled *Ornithodorus)* ticks can survive for a long time without food and at a low humidity. A thin layer of wax-like substance in the epicuticle and the capability of absorbing moisture by closing the spiracles allows *Ornithodoros* to remain alive under unfavorable conditions. About 15 species of this tick have been proved to carry borreliae.

Ornithodoros feed exclusively on blood. They may ingest amounts equivalent to 2 to 6 times their own weight.[438] Saliva reaches the capillaries in the skin of the bitten animal. Towards the end of the feeding the contents of the gut are evacuated through the mouth. There is no rectal outlet, only a urinary pore with a rectal bladder and two Malpighian tubes. The urinary pore is often called a rectal pore but feces are not discharged through it. Water is excreted through the coxal glands. The pressure of the engorged gut seems to aid in this process. There may be copious coxal fluid excretion (as in *O moubata*) or only a drop which may not appear at all while the tick is in contact with its host during feeding. Normally, nymphs feed more often than adult ticks.

Figure 5. Head of louse, Pudiculus humanus.
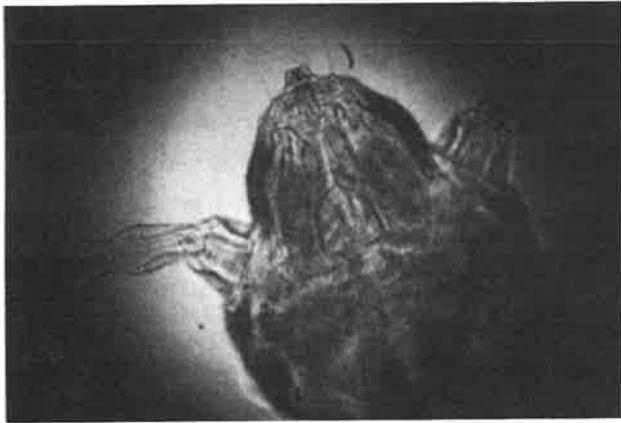
Figure 7. Male louse, Pediculus humanus (corporis).

Figure 6. Claws of human louse, Pediculus humanus.
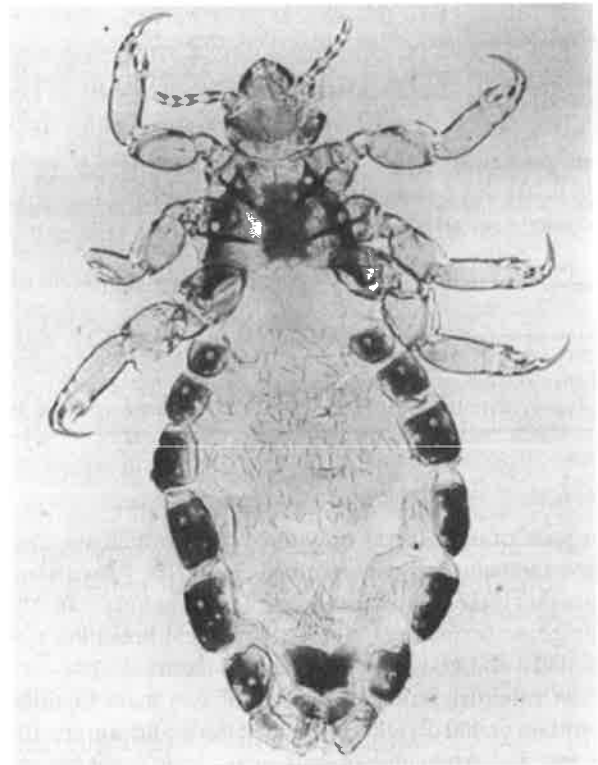
Figure 8. Human louse, Pediculus humanus (corporis), *female. Slide preparation.*

After feeding, *Ornithodoros* leave their host.

Sautet[624] summarized the natural animal hosts of *Ornithodoros*. His studies and those of Mooser[501] and others show that *O moubata* and the human body louse are primarily anthropophilic, whereas other ticks are parasitic principally on other animals, mainly rodents and insectivores.

There are numerous animals on which certain species of *Ornithodoros* feed. The feeding usually takes 10 to 30 minutes, seldom 1 hour or longer according to the species of tick. The bite of some species is painful, whereas others produce analgesia. *Borrelia* transmitting ticks usually do not have a painful bite, and feed for a relatively short time, which affords them some safety from being scratched or shaken off by the host. Baltazard et al,[56] discussing host-vector relationship of ticks, stated that young animals are better hosts because they are unable to rid themselves of the tick with ease. Considering the greater susceptibility and higher mortality rate of young rodents when infected with borreliae, and the willingness of

Ornithodoros to feed also on young dead animals, the tick-rodent relationship in a given ecosystem results in the survival of the tick but in a diminishing number of their young hosts. The time of the emergence of hungry nymphs often also coincides with that of new wild rodent litters, which further contributes to the effective survival of *Ornithodoros*.

As in the louse, the borreliae penetrate into the celomic cavity of the tick. First small, thick, and corkscrew-like forms are seen, as well as thin, elongated borreliae. They

have a predilection for the central ganglion, the two coxal and salivary glands, and the genitals including the gonads. This results in transovarian transmission which does not take place, however, in all instances. Borreliae pass with the eggs but not all larvae become infested.[7] Details were studied by Aeschlimann[8] and Wagner-Jevseenko.[708] The borreliae appear to penetrate the follicular layer around the ovules, pass through the surface of the eggs, and reach the yolk through the protoplasmic cortex. The number of infested eggs varies according to the species: up to 100% *O turicatae*, 80% *O moubata*, but less than 2% *O hermsi* eggs will become infested.[222,303] Borreliae multiply during larval development and reach the salivary glands, so that first-instar (F_1) nymphs are already infested.

The organotropism is probably of chemical nature. Bruen and Blatter[104] believed that it might be due to an oligosaccharide such as glucose.

Tick vectors and their life cycles have been discussed by Davis[219,220] who listed in the Americas *O hermsi* Wheeler, Herms, and Meyer 1935 and *O parkeri* Colley 1936 from the Western United States; *O rudis* Karsch 1880 from Colombia, Venezuela, and Panama; *O talaje* Guérin–Ménerville 1849 from the same area and also Argentina; and *O turicata* Duges 1876 from the United States and Mexico, among the *Borrelia*-carrying ticks in the Western Hemisphere. Desportes and Campany[237] enumerated *O tholozani* Laboulène and Méguin 1882 and other ticks of Asia Minor and Central Asia. Enigk and Grittner[257] presented a general survey of ticks and their biology.

Baker and Wharton[41] in their monograph suggested that borreliae evolved with Acarinae. This hypothesis implies that borreliae were primarily symbionts or parasites of ticks, specialized in *Ornithodoros* species by genetic evolution and adaptation, and invade mammals only by chance. This theory can be brought in accord with that of Nicolle and Anderson[520,522] that ticks conserve and lice propagate borreliae, even though the latter authors believed that borreliae originated as parasites of small mammals. which does not seem plausible from today's vantage point.

The monographs of Baker and Wharton[41] and of Arthur[26] on ticks, that of Cooley and Kohls[200] on Argasidae in the Americas, the list by Hoogstraal of ticks in North Africa[371] and by Galouzo[290] in Central Asia, the review by Anastos[15] of Ixodides in the USSR, and numerous special communications of the group led by Geigy, Burgdorfer, and Aeschlimann on African ticks (*vide infra*), together with the reviews by Nicolle et al[529] and by Heisch,[343] should be consulted for details.

Other and equally important reviews of *Ornithodoros* and tick-*Borrelia* relationships include that of Bohls,[89] who listed *O venezolensis* among the tick vectors in the Americas, and described their habits and habitats as fol-lows. In the Western United States and in Texas, *Ornithodoros* like to establish themselves firmly in caverns, in Southwest Kansas in burrows of prairie dogs, in the state of Washington in owl burrows, and in Southwest Texas in rodent burrows. Domestic animals do not appear to be hosts of these ticks in the United States but *O venezolensis* and *O talaje* are often found in or near human habitations, *O turicatae* in Texas sometimes under houses, and *O hermsi* in or near summer cabins at relatively high altitudes, above 5,000 feet. Bohls pointed out that a tick species may be infested but does not have to cause human infection. Also, the tick population may be so lightly infested that samples collected from it may not reveal the presence of borreliae.

Baltazard and his group[53] observed different conditions in Asia and Africa. *O erraticus* was found feeding on rodents in burrows. These authors listed studies on the so-called crocidurae sub-group of borreliae and discussed Central Asian strains. Cooley[199] stated that *O erraticus* Lucas 1849 dwells in pig sties and burrows, and may have several hosts including frogs *(Bufo pantherinus). O moubata* Murray 1877 lives with man and domestic animals in Africa. *O savignyi* Audouin 1826 in Africa and Asia keeps similar company. Not all authors agree, however, that *O savignyi* is an effective carrier of borreliae. *O cholodovskvi* Pavlovsky 1930 lives in Turkestan; *O lahorensis* Neumann 1918 in an area from India to Turkey and Palestine, with domestic and wild animals, as well as with man; *O tartakovskyi* Olenev 1931 in Central Asia with *Tatera* and other animals; *O tholozani* Laboulbène and Méguin 1882 (synonym *O papillipes)* in Central Asia and Iran with man, camels, chickens, and in rodent burrows.

Hindle[365] pointed out that *B normandii* may be identical to *B hispanica* de Buen 1926; *B sogdiana* Nicolle and Anderson 1928 and *B uzbekistana* Picoul 1928 may be the same as *B persica* Dshunkowsky 1913. He also identified the crocidurae Leger 1917 subgroup with *B duttonii* Novy and Knapp 1906 which is contrary to serologic, epidemiologic, and clinical experience, if we speak in terms of species of *Borrelia* at all.

Davis[222] in his review maintained the theory of tick specificity but emphasized that numerous exemptions are possible, as *B microti* being transmitted both by *O lahorensis* and *O canestrini*.[236] He also pointed out the confusion existing with respect to American ticks. *O rudis* is synonymous with *O venezolensis (O venezuelensis)* and is the vector of *B venezolensis* Brumpt 1921 and *B neotropicalis* Bates and Saint–John 1922. *B neotropicalis* has been reported also from Panama where it is transmitted by *O rudis*. *O talaje* bites animals but not man. Clark[182] believed that it transfers the infection only from animals to animals, whereas *O rudis* takes part in the animal-vector-man-animal cycle. Davis also discussed the role of coxal fluid in
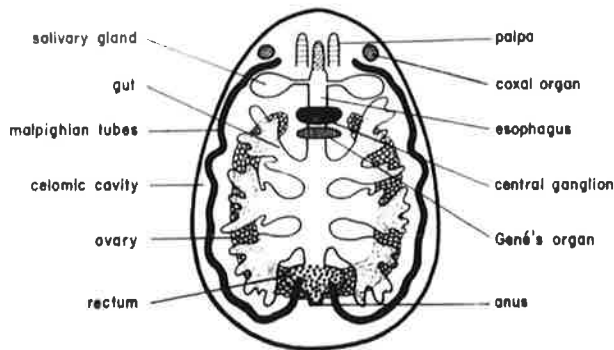
Figure 9. Schematic section through an Ornithodoros.

the transmission of borreliae. Coxal fluid is expelled by the feeding tick as it becomes engorged with blood. It contains and transmits borreliae to the wound caused by the bite of the tick. Many ticks, like *O turicata*, and particularly nymphs, do not expel coxal fluid but infect through their bite. This will be discussed in detail later.

Horsfall[374] added to the list of ticks carrying relapsing fever *O brasiliensis* that harbors *B brasiliensis*, *O tholozani* as a carrier of *B babylonensis*, *O normandi* as a vector of *B normandii*, and *O dugesi* as a carrier of *B dugesii*. It is doubtful, however, that *B babylonensis* and *B normandii* represent independent species. Horsfall emphasized that *O erraticus* has two subspecies, namely, *O erraticus erraticus*, the large form, the vector of *B hispanica*; and *O erraticus sonrai*, the small form, carrying members of the crocidurae subgroup. *O e erraticus* likes some moisture, *O e sonrai* dry burrows.

Nicolle and Anderson[524] stated that *Ornithodoros* have to be infected in the nymphal stage to become effective vectors.

Ornithodoros, therefore, may propagate borreliae from one generation to another. The borreliae may be transmitted to man and animals through the coxal fluid which contaminates the site of the insect bite, or, principally by young nymphs and in North American species also by adult ticks, through the salivary glands. The tick does not have to be injured, as does the louse, to transfer *Borrelia* to man or animals.

Observations on individual *Ornithodoros* and *Borrelia* species that are important from the medical point of view follow.

Specific Ticks and Borreliae
Ornithodoros moubata and Borrelia duttonii

Ornithodoros moubata, the eyeless tampan, has four subspecies, *O compactus*, *O apertus*, *O porcinus*, and *O porcinus domesticus*.[714,715] It carries *B duttonii*. Its principal homes are West and East Africa. Manson and Thornton[467] stated that the infection is severe, with many

complications, in Europeans; severe but with few complications in the indigenous population. Moreover, these authors believed that several strains of *B duttonii* may be carried by *O moubata*. The organisms were described as 20 to 35 μ long, about 0.25 μ wide, with 5 to 9 spirals. Short and longer forms were common. Of 600 *O moubata* ticks, 29% harbored borreliae. Dubois[247] also found different serotypes of *B duttonii* in this tick.

Feng and Chun[270] could observe borreliae for a long time (11 days) in the stomach of *O moubata* after a blood meal, but the borreliae appeared in the celomic fluid and in the salivary glands, neural ganglion, and coxal glands of the tick as early as within 6 hours. Multiplication of the borreliae took place in the organs and celomic cavity, by transverse division. No borreliae were seen by these authors in the Malpighian tubules or in the feces. The same writers stated later[271] that the central neural, ganglion is the predilection site of *B duttonii*. After long starvation, the borreliae may disappear from the coxal fluid and ovaries.[428] The tick can fast long, even a year or more, and remain alive. Adults usually feed every 6 weeks, nymphs every 2 to 3 weeks.

The subspecies of *O moubata* usually prefer a single animal host for their blood meal.[309,709] The blood meal lasts 20 to 30 minutes but the blood is digested very slowly. Precipitin tests showed its presence 7 months after feeding.[721] The blood passes through the esophagus into the midgut and its diverticula which are relatively spacious and have quite elastic walls.

Molting takes place between meals. The lifespan of *O moubata* is 2 years, on the average. They withstand severe dehydration.[110,134]

Transovarian transmission of borreliae is the rule in *O moubata*. Males may harbor *B duttonii* in the genital organs but do not transfer them by the sexual act.[313] Freshly fed females mate, burrow into the sand, and lay 30 to 80 eggs of about 0.6 mm diameter.[303] The six-legged larvae hatch best at about 25° C. Six 8-legged nymphal stages follow, then the adult stage, within about 8 months. About 40% to 60% of the offspring are infected with *B duttonii*.[313] This proportion may vary, however, from one village to another, with an average of 15%.[303] The salivary glands are irregularly infested in adults but contain many borreliae in the nymphs.[699] Therefore, young adults and nymphs propagate the infection principally through their saliva, whereas adults spread it through the coxal fluid.[302] The tick becomes infective 5 to 6 days after feeding on blood that contains *B duttonii*. The first nymphal instar is the most effective transmitter also because of its rapid mobility. Geigy and Burgdorfer[129,300,301,302,306] demonstrated the efficiency of *O moubata* in maintaining the infection without a vertebrate host.

O moubata is found only in Africa but not in the dry desert, the rain forest, or above an altitude of 2,000 meters (6,600 ft.). *O moubata* prefers fine loam on which African huts are built and in which man and fowl live together.[712] *O moubata* does not climb well, and likes to bury itself in the soil a few centimeters deep, particularly near dry places where people usually sit. It may be found in cracks, holes, and crevices of the mud floors and sometimes also in the grass walls. *O moubata* appears to be domesticated but is still found in burrows of wart hogs, porcupines, hyenas, aardvarks *(Orycteropus),* and bats.[344] Walton[711] believed that its original hosts were these animals, and that it came to human habitations with hunters. This tick adapts itself easily to other hosts and may learn to bite cats, dogs, and pigs which, however, are not susceptible to *O duttonii* infections.

O moubata emerges from its hiding places after sunset. It is attracted to the bodies of people sleeping on the floor, and to fire. All stages, except eggs and larvae, take blood meals.

Details of the transmission of *B duttonii* were worked out by Geigy and his coworkers[301,302,306,311,312] and others. Burgdorfer[129] observed that borreliae shorten from about 22 μ to 10 μ, gather at the stomach wall, and penetrate it. No borreliae are present in the stomach two weeks after the infective meal. The hemolymph of the tick sometimes harbors borreliae as early as the second day. Multiplication takes place in the celomic cavity. The coxal glands and the central ganglion may be invaded even on the third day, the Malpighian tubes somewhat later, but their lumina remain free from borreliae. There is a crossing of borreliae between the hemolymph and the organs for which it has predilection. The process of invasion of the *Ornithodoros* is slower at 20°C than 30°C environmental temperature. Varma[699,700] found the central ganglion infected in all instances, whereas the coxal glands harbored borreliae in 75%, and the Malpighian tubules in 25% of the examined ticks. Sarasin[622] believed that *B duttonii* can enter any firm organ of *O moubata* but that it lives always in intercellular spaces except in the oocysts. Aeschlimann[8] studied the transovarian transmission. *B duttonii* enters oocysts early. The borreliae are located in the intercellular spaces but become intracellular after the tunica propria is formed.

Grün[327] observed that *O moubata* did not acquire *B duttonii* when this organism became nonpathogenic after numerous animal passages. The same strain of *O moubata* could, however, transmit *B hispanica.* Some loss of virulence of *B duttonii* in the 5th generation of *O moubata* was described by Aeschlimann.[9] The transstadial and transovarian development, including the problem of filial variations in relation to the number of infective progenies, was summarized by Burgdorfer and Varma.[130]

Colas-Belcour et al[189] studied ticks from Madagascar which were not naturally infected and found that about 50% of these *O moubata* will acquire *B duttonii* if given a blood meal containing this *Borrelia.*

Further problems of the *O moubata-B duttonii*-animal-man relationship were investigated by several workers. Heisch and Grainiger[350] found that *O moubata* living in rodent burrows is not a reservoir for human infection because of the rarity of contact between man and tick in the areas inhabited by wild rodents. Arboni[23] stated that *O moubata* may live with guinea pigs, rabbits, pigeons, other fowl, and horses, but not uniformly with pigs. Fresh cattle, goat, and sheep serum killed *B duttonii* but this activity of the serum was destroyed by heating. The origin and quality of the antibodies in these sera are open to question. Geigy and Mooser[305] studied wart hogs, the natural host of a variant of *O moubata* without being able to find proof of infection.

Geigy and Herbig[308] stated that *O moubata* does not have a known and useful natural host. On the other hand, Walton[712] observed *O moubata* in the Digo district of Kenya near the border of Tanganyika but only in houses that also sheltered fowl. The ticks harbored *B duttonii* but human cases of infections were rare. Walton considered this an example of an animal reservoir without overspill into the human population. It is possible, however, that the human inhabitants had acquired immunity during undiagnosed episodes of infection in their childhood, or that the ticks preferred to feed on chickens. Schwetz[633] also found infected *O moubata* but no borrelemia in human beings who lived in the same huts in various localities between Lake Kivu and Lake Albert in the Congo. He explained this as a state of immunity following past infection in the local inhabitants.

O moubata is an effective vector. It was shown to be able to carry *B hispanica for* many months in laboratory experiments[296] but not the East African strain of *B recurrentis.* Baltazard et al[54] had moderate success in transmitting *B crocidurae* to it. *B turicatae* lived in *O moubata* in the laboratory for a long time but the tick did not transmit the *Borrelia.* Thus *O moubata* appears to be carrying only *B duttonii* in nature.

Ornithodoros erraticus erraticus and Borrelia hispanica

Ornithodoros erraticus erraticus or the "large" *O erraticus* carries *Borrelia hispanica.*

Several strains of *B hispanica* have been described. We believe, however, with Nicolle et al,[528] that both the so-called Moroccan and Mansourian (Bou Znika) strains are *B hispanica,* as well as those labelled as Tripoli, Portuguese, Peloponese or Greek, and Normandian (South Tunisian) strain. Moreover, we include the atypical Syrian and Algerian strains[511,625] also in this species.
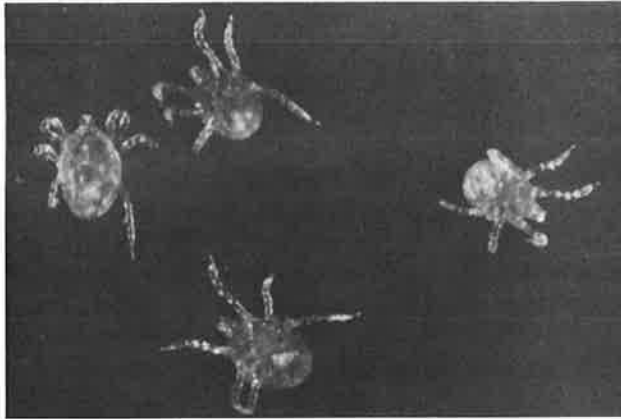
Figure 10. Ornithodoros tartakovskyi, *nymphs. Photograph by Dr. T. C. Orihel.*
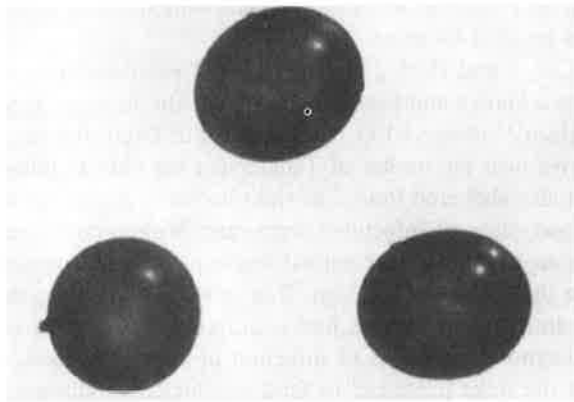
Figure 11. Ornithodoros tartakovskyi *eggs.*
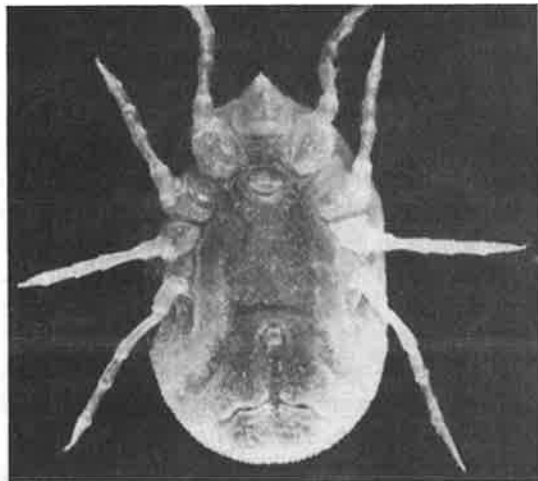
Figure 12. Ornithodoros tartakovskyi, *adult females, ventral view. Photograph by Dr. T. C. Orihel.*

The disease caused by *B hispanica* is usually mild[230] whichever subtype is causing it. Exceptions are not rare, however.

According to Muñoz Cosin[510] *O e erraticus* is a nocturnal tick. It feeds for 15 to 20 minutes. It tolerates lack of food well even at 28°C and 30°C if the humidity is high enough. *O e erraticus* apparently requires somewhat higher atmospheric moisture than does *O moubata.* The borreliae are transmitted to the host after feeding, with the coxal fluid, but infection by the bite of the tick is also possible.[277]

O e erraticus appears to have followed the route of the Moslem conquest, along the Mediterranean Littoral through North Africa to Spain. It established itself also in Portugal and Greece. The Moroccan strain was found in ticks in burrows of porcupines[230]; others also in the dwellings of gerbils, hedgehogs, jackals, and wild rats[54]; Muñoz Cosin[510] in addition to the foregoing observed the tick living with foxes, weasels (*Mustela vulgaris*), and pigs. It seems that pigs do not become infected with *B hispanica* but may disseminate the tick. Neither is there an agreement that foxes are susceptible to *B hispanica* infection. Baltazard[45] confirmed the findings of Nicolle and his coworkers in Tunisia, and isolated *B hispanica* from wild rats, man, and *O e erraticus* in Casablanca. Delanoë[232] found hedgehogs infected, as well as young jackals. Older jackals appeared to have antibodies against *B hispanica.* Young porcupines were also susceptible but grown animals had measurable antibodies.[231] Similar results were observed in weasels.[233] Wild rats were found infected in Greece.[141] Nicolle and Anderson[527] examined closely the relationship of hogs to relapsing fever in North Africa. *B hispanica* did not circulate in their blood. Even though they may act as host to *O e erraticus*, they did not appear to be important as animal reservoirs of relapsing fever. A survey of cave-dwelling bats in Spain[515] showed 4 susceptible species (*Miniopterus schreibersii, Myotis myotis, Rhinolophus euryale,* and *R hipposideros*).

Mathis et al[480] commented on the observation that tick-borne relapsing fever in Tropical Africa is due to *O moubata* with the exception of Dakar, where *O erraticus* was also found. Boiron[95,96,97] investigated this problem and found that 18.5% of the blood and 40% of the brain or spleen specimens from 512 rodents belonging to 9 species were infected. *Cricetomys gambinus* and *Rattus rattus alexandrinus* were most frequently infected, and *O e erraticus* was found on them. More cases were in modern Dakar than in the adjacent township of Medina. The number of rat burrows in the hard soil of Dakar was higher than in the light sandy soil of Medina. Also, *O e erraticus* appeared to find more feasible moist shelter in Dakar. Boiron also stated that hedgehogs and some reptiles may serve as hosts but man encounters *O e erraticus* easily in Dakar.

Not only *R r alexandrinus* in Dakar has been found to be an important reservoir but also other rats have been implicated, as the gray rat (*Ratus norvegicus*) in
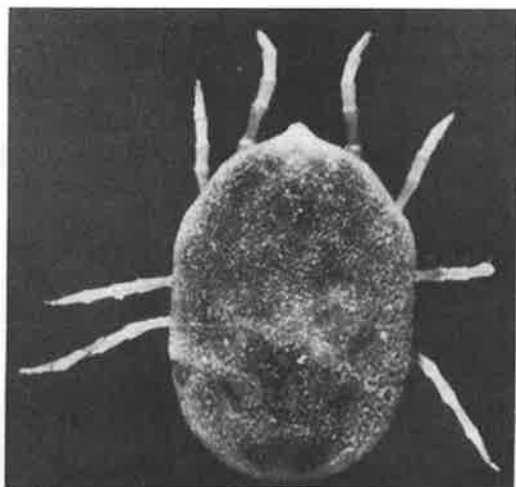
Figure 13. Ornithodoros tarakovskyi, *adult female, dorsal view.* Photograph by T.C. Oribel

Figure 15. *Dorsal (left) and ventral (right) view of* Ornithodoros tartakovskyi, *male.*


Figure 14. Ornithodoros tartakovskyi, *male, ventral view. Photograph by Dr. T. C. Orihel.*

Figure 16. *Holes in fallen trees, where* Ornithodoros turicata *were found.*

Casablanca[85] and in Algeria.[373,465] The role of the porcupine in Africa was more closely investigated by Nicolle et al.[530] They concluded that porcupines may be reservoirs of *B hispanica* but live away from human habitation and roads and are, therefore, not an important source of human infection.

O erraticus was found feeding on crabs when mammalian blood was not available. The crabs were resistant to borreliae and their blood remained free of them.[50]

Jahnel[382] studied the fate of *B hispanica* in hibernating dormice. The borreliae disappeared faster in the cold than at room temperature. If the animals were infected during hibernation, *B hispanica* was recovered over longer periods than when the infection took place before hibernation.

B hispanica, Tripoli strain, was maintained by Nicolle

and Blaizot[531] through 12 rabbit passages without loss of virulence. Chorine and Colas-Belcour,[167] however, found gradual diminishing of virulence after human passages, at a slower pace also in rodents. The incubation period also becomes longer.

Brumpt[123] described *B babylonensis* from *O asperus* which, however, was easily transmitted by *O persica.* It caused many relapses in guinea pigs and the organisms in the blood were as numerous as in *B hispanica* infections. *O tholozani* and *O erraticus* could not be infected with this strain. *O coriaceus* (which does not transmit any known *Borrelia)* could not transmit it. This strain, unfortunately lost by now, may be a variant or a transient organism, especially since *O asperus* was shown to transmit *B persica* in the laboratory. The geographical location of the finding of this *Borrelia* tips the balance in favor of *B babylonensis* being a strain of *B persica.*

Experimentally, *B hispanica* has been transmitted to *Argas persicus, O moubata, O savignyi, O tholozani, O turicata, Haemaphysalis inermis, Pediculus humanus,*

Figure 17. Rodent burrows harboring Ornithodoros turicata.

Figure 18. Semidesert in Asia, harboring Ornithodoros erraticus *with small rodents.*

Figure 19. African forest with rodent burrows. Photograph by Dr. Clyde Jones.

Hemstopinus suis, and *Xenopsylla cheopis* by Muñoz Cosin[510] and to *O moubata* by Kudicke et al.[428] It was isolated from the dog tick *Rhipicephalus sanguineus* in Greece.[141] Davis and Mavros[227] observed its survival in *O nicollei* for 5 years and noted that it was transmitted to the F_1 generation in this tick. Baltazard et al[54] transmitted *B hispanica* by *O savignyi, O moubata,* and *Rhipicephalus sanguineus* in the laboratory. It appears that this *Borrelia* can be transmitted in the laboratory by many genera and species of insects. It is questionable if the same holds true in nature.

Ornithodoros erraticus sonrai and the "Crocidurae" Subgroup

Ornithodoros erraticus sonrai or the "small" *O erraticus* is the vector of a group of borreliae that are only moderately or not at all pathogenic for man, but may cause disease in rodents.[60,480] The first member of the group, *B crocidurae,* shows some crossimmunity with *B duttonii.*[525] Some experts consider *B crocidurae* identical to *B merionesi* and *B microti,* whereas *B dipodilli* of Kenya is regarded as a distinct strain.[303] Baltazard et al[53,60] reported this group in Senegal, from the countries along the Southern Mediterranean, Iran, and East Africa. There is considerable disagreement concerning the status of the individual strains of the crocidurae subgroup. One of the principal reasons for the separation of this group was the difference in the tick vector. *B crocidurae* was isolated from the wild shrew (*Crocidura stämpfii*) in Dakar by Leger in 1917. *B crocidurae* is more virulent than the other members of the subgroup and often fatal for rats (*Cricetomys gambianus, Rattus rattus alexandrinus, Ratus norvegicus,* and *Arvicanthis sp.*), rarely for *Epimys, Rattus* and *Mus musculus.*[478] Local people in Dakar appeared to be immune to *B crocidurae.*[481] Baltazard et al[62] isolated it in Turkey, and found that this strain causes prolonged disease in guinea pigs but only weak or subclinical infections in man. Colas-Belcour[188] considered *B crocidurae, B microti,* and *B merionesi* immunologically interrelated but different from *B hispanica.* Dirk van Peenen[241] studied borreliae in *O e sonrai* that were found with Nile rats (*Arvicanthis niloticus*) by Davis and Hoogstraal in Egypt. Newborn rats and mice seem to be susceptible to *B crocidurae* but adult guinea pigs are not. Some investigators[195,303] emphasized that *O e erraticus is* not hospitable to *B crocidurae* but that this organism was transmitted by the ovum to the F_1 generation of that tick subspecies. Vertebrates were infected during feeding, through the saliva of the infected tick. *O e erraticus* did not carry other members of the crocidurae subgroup.

B merionesi was isolated in South Morocco by Baltazard. It was studied by Baltazard's group (see above) and was found to be different from *B duttonii* and *B his-*

panica. It frequently caused fatality in rats and hamsters. Blanc and Maurice[84] found it nonpathogenic to man.

B microti was isolated from *Microtus mistacinus* and from *Tatera indica,* as well as from rats and *Cricetulus migratorius* in Hessarak and other localities in Iran by Rafyi.[592] This strain did not infect guinea pigs but caused some disease in man. Delpy[234] studied this organism in Iran and found 35% of *Tatera,* 14.8% of *Cricetulus,* and less than 12% of other rodents, such as *Nesokia, Mus,* and *Microtus,* infected with it. *Meriones shawi* also carried this *Borrelia.*

B dipodilli was described by Heisch[342,343] from pygmy gerbils *(Dipodillus gerbillus)* in Kenya. It was mildly pathogenic for rats, mice, monkeys, and young rabbits, but not for guinea pigs and man. Heisch believed that *B dipodilli is* related to rodents in North Africa and the Middle East, and posed the question whether or not *B duttonii* has evolved from it because of the belief that *B duttonii* was originally a rodent-oriented *Borrelia.*

In laboratory experiments, *B crocidurae* could be transmitted to the human louse but *P humanus* did not transfer this organism to man.[95,98,331] Monkeys have been found susceptible to *B crocidurae* as well as to *B merionesi* and *B dipodilli.* The last (as well as *B duttonii)* was able to infect the monkey louse, *Pedicinus longiceps,* and transmit the borreliae among nonhuman primates. The monkeys did not become seriously ill. This may point toward an additional life cycle of the members of the crocidurae subgroup. Ticks other than *O e sonrai* are not very hospitable to this group of borreliae.[594] Therefore, adjustments, accommodation, and adaptation must have taken place before the present tick-reservoir of *B duttonii* could develop, if the theory of Heisch is correct.

Ornithodoros tholozani (papillipes) and Borrelia persica

Ornithodoros tholozani (synonym: *Ornithodoros papillipes)* appears to be identical also to *O crossi* which carries the Kashmir strain of *Borrelia.* It may have several subspecies. *O tholozani* carries *B persica. B persica* was described by Dschunkowsky in 1913.[245] The domain of *O tholozani* spreads from Lybia through Western Egypt, the Arab countries, Cyprus, and Turkey, to India and Central Asia. It is possible that *B uzbekistanica* and *B sogdiana* are variants of or identical to *B persica.*[444]

O tholozani has one larval and 3 to 4 nymph stages. Each one of these stages feeds on a vertebrate at least once but the adults take a blood meal more often.[691] Hereditary transmission is the rule[6,42,506,691] but Bourgain[108,580] failed to prove it. He suggested that the nymphs become infected during their blood meals.

O tholozani lives for a long time. Bourgain[107,108] reported that nymphs may live 5 years, if no food is available that is a prerequisite for molting; adults live about 7 years.

Adler et al[5] and others showed that *B persica is* transmitted by the bite of *O tholozani* and not by the coxal fluid. Slavina[651] found 2% of ticks collected in their natural habitat infected with *B persica.*

It appears that infected nymphs[108] and adult ticks[42] may lose borreliae during their lives, and the virulence of the strains may decrease in the ticks. Pirot and Bourgain[580] stated that infected ticks apparently die sooner than noninfected individuals. This problem should be further investigated since Balashov[42] saw an increased infection rate in successive tick generations, from 11% to 47%.

O tholozani lives in caves and burrows of small animals in Central Asia in oases along the edges of the woodlands,[563] and also with man. It was found in the huts of regimental followers in Qetta near the Afghan border,[114] and collected in abandoned piggeries,[691] often near fowl and camels.

Lice, especially those adapted to feeding on suckling rabbits, will take up *B persica.*[161]

O tholozani has been infected artificially with *B sogdiana*[21] but this experiment may have involved merely the transmission of the same strain or its variant. *B recurrentis* can survive in *O tholozani*[656] but lice fed on patients infected with *B persica* do not transmit this organism. Rafyi and Maghami[594] were able to infect *O tholozani* with *B hispanica* and *B microti.*

O lahorensis has been thought of as a possible vector of *B persica.* Pavlovskii[563] and others proved that this is not possible because *O lahorensis* does not bite man and even though it acquires human-pathogenic borreliae by feeding on rodents in the laboratory, the borreliae die off in it rather quickly.

Babudieri[36] encountered two possibly distinct strains of *B persica* in Jordan. One, the rural form, caused disease in sheepherders, migrants, road builders, and other persons who rest in caves with dry and sandy floors harboring *O coniceps.* The peak number of infections was in the winter. The other, the urban form, appeared in badly maintained and ventilated houses with earthen floors. The greatest number of infections was observed in the summer. Babudieri believed that *O tholozani* is the vector in urban areas, where domestic rats could serve as a reservoir of the infection. It is still being debated, however, whether *O coniceps* is a vector of borreliae *(vide infra).*

Ornithodoros tartakovskyi and *Borrelia latyschewii*

Sofiev[655] described *Borrelia latyschewii* from *Ornithodoros tartakovskyi.* This *Borrelia* was isolated from gerbils *(Rhombomys opimus* and *Gerbillus eversmanni)* as well as from *O tartakovskyi* caught in burrows of rodents. It causes mild disease in man, often only a one-day fever, and one or two attacks in mice but not in

Figure 20. African hut in which Ornithodoros moubata *thrives*. *Phtograph by Dr. Clyde Jones.*

Figure 21. Huts with wooden walls (foreground) and clay walls (back) in Africa, where Ornithodoros moubata *has been found. Photograph by Dr. Clyde Jones.*

Figure 22. Huts in which man and fowl live together in Africa, where Ornithodoros moubata *has been found. Photograph by Dr. Clyde Jones.*

rats, guinea pigs, and dogs. The number of borreliae is low in the blood even after successful infection. Sofiev and Leitman,[656] analyzing tick-borne and louse-borne relapsing fever in Central Asia, pointed out that this *Borrelia* cannot be transmitted by lice to man in that region, nor can *O tartakovskyi* propagate *B recurrentis*.

The latter *Borrelia* will, however, survive in *O tartakovskyi* for 4 months.

Baltazard et al[55] isolated *B latyschewii* from *O tartakovskyi* in Iran, near Meshed. This strain did not infect adult guinea pigs which is unusual in Old World tick-borne borreliae. The organism was slightly pathogenic for man but was not found in nature in rodents with which the tick lived. It appeared to prefer the burrows of merions and other wild rodents, tortoises, lizards, and toads. Wild rodents such as *Meriones, Microtus,* and *Rhombomys* could be infected in the laboratory.

O tartakovskyi exudes coxal fluid some time after it has left the host on which it fed. This, perhaps, explains the small numbers of borreliae transmitted into the animal by the bite of the tick.

The natural reservoirs of the Central Asian and the Caucasian parts of the USSR were studied by Pavlovskii.[565] *Dryomys nitedullus*, a small, widely distributed rodent, is readily infected with borreliae and carries them for a month or longer. The principal foci were not associated with forests but with oases along the base of the mountains. Petrishcheva[571] investigated the duration of natural foci in Turkmenia. Not only *O tartakovskyi* but also *O tholozani* and *O neerensis* were found. Caves inhabited *by Hystrix hirsutirostis* were studied but a change of the inhabitants of the burrows did not reduce the danger of infection for man. Of 13 foci, 8 were active 16 to 19 years, 2 for 21 to 29 years, and 3 for 30 years. This bears witness to frequent and sustained transmission cycles. Chickens, pigeons, gold fish, and 19 bird and reptile species were found resistant to *B latyschewii* and *B persica* in the same area.[496] No borreliae were found in 1144 reptiles caught in homes. Thus, reptiles cannot be considered a reservoir of Central Asian tick-borne relapsing fever borreliae.

O tartakovskyi intrudes into animal sheds, cattle serving as a source of its blood meals. It may also invade floors and the lower parts of walls, hiding in cracks, holes, and crevices. Moreover, when new lands are being opened for agriculture, people may come into contact with *Borrelia*-carrying ticks, and the cycle tick-rodent-tick may be extended to tick-rodent-tick-man-tick-man-(rodent)-tick.

Ornithodoros neerensis is also supposed to carry *B latyschewii*.[566,587] It is not known if the two tick strains are identical or related.

Orinthodoros verrucosus and Borrelia caucasica

Ornithodoros verrucosus, the vector of *Borrelia caucasica,* lives in burrows of merions *(Meriones erythrourus caucasicus), Apodemus sylvaticus,* and *Mus musculus.* The disease in man may be severe and consist of 10 to 15 relapses within 3 months. Chubaryan[171] found jer-

Figure 23. Ruins in the Mediterranean region where Ornithodoros erraticus *was found.*

Figure 25. The home of Ornithodoros tartakovskyi.

Figure 24. Ornithodoros tholozani *was found in this building.*

Figure 26. Ornithodoros talaje-*infested building.*

boas *(Alactaga elater, A williamsi)* susceptible to the infection. Laboratory rodents acquire mild disease or are only slightly susceptible to this *Borrelia.* Guinea pigs develop several attacks. To the knowledge of this author, *B armenica* has not yet been studied outside the USSR.

Ornithodoros zumpti and Borrelia tillae

Geigy,[303] when considering *Borrelia tillae,* regarded it as a phylogenetic problem. It may have evolved from human-borne *B duttonii* by progressive specialization because *O moubata* does not feed on domestic rodents, and wild rodents are resistant to *B duttonii.*

Zumpt[748] described the tick vector in 1959. Zumpt and Organ[749] reported on *Borrelia tillae* isolated from *O zumpti* Heisch and Guggisberg living in holes of the South African field vlei rat *(Otomys saundersiae).* White mice and the multimammalian rat *(Rattus natalensis)* are highly susceptible to the infection. Patas monkeys *(Erythrocebus patas)* and adult guinea pigs, as well as some rabbits, were refractory to the infection. Geigy and Aeschlimann[305] succeeded in transmitting *B tillae* to *O moubata* but serologic tests and electron microscopic studies showed that *B tillae* is not indentical to *B dut-*

tonii. Moreover, *B tillae* was not pathogenic for white rats, hamsters, and merions. On further investigation, *B tillae* was isolated also from the brains of the four-striped rat *(Rhabdomys pumilio)* and *R natalensis.* Heisch and Harvey[354] confirmed these findings. One may expect further developments in the study of this interesting *Borrelia.*

Relapsing Fever Vectors and Borreliae in the Americas

Calero[137] surmised that the American relapsing fever strains are tick-adapted *B recurrentis.* Brumpt[118] believed, however, that American borreliae did not become tick-borne after the Spanish Conquest or after the settlement of the West commenced when louse-borne relapsing fever was introduced. Tick-borne borreliae were probably already present in the western mountain ranges and lands when the first immigrants arrived. Kemp et al,[407] Wynns and Beck,[741] Beck,[69] Wheeler,[727] Davis,[271,218] and others gave detailed accounts of the borreliae and their vectors in the Americas.

Ornithodoros turicata and Borrelia turicatae

Ornithodoros turicata Dugés 1876 is the vector of *Borrelia turicatae* Brumpt 1933. Kemp et al[406] observed that larvae and nymphs feed 10 to 30 minutes, adults for hours and even for two days. The borreliae are transferred by the bite of this tick. Coxal fluid is secreted after meals but does not contain borreliae. *O turicata is* easily infected with *B turicatae*. Practically all adults carry it and propagate the *Borrelia* at least to the F$_5$, generation.[221] Francis[278,279] observed that starving *O turicata* may survive for 5 years.

O turicata has been found in Canada, the Western United States as far east as Kansas, in Mexico, and in South America. Caves, especially those which are entered by goats and sheep, and burrows of rodents, such as those of field mice in Central Mexico,[483] and burrows of owls and snakes are the habitats of *O turicata*. It is found under houses in Texas[89] and it is becoming domesticated in Mexican huts and animal barns.

The central ganglion of the tick is not as hospitable to *B turicatae* as it is to *B duttonii*.

Brumpt and Brumpt[124] and Mazzotti[483] demonstrated that mice, rats, guinea pigs, cotton rats, rabbits, pigs, dogs, cats, and foxes can be infected with *B turicatae* in the laboratory but not hedgehogs and dormice. The virulence for guinea pigs is low.

O turicata cannot transmit *B duttonii, B venezolensis,*[118] or *B dugesii* Mazzotti 1949.[228]

Ornithodoros parkeri and Borrelia parkerii

Ornithodoros parkeri Cooley 1936 transmits *Borrelia parkerii* Davis 1942. It lives in the western region of Canada and the United States but not in Mexico, in caves and burrows inhabited by ground squirrels, prairie dogs, and burrowing owls.[384] It only infrequently encounters man. The infection is transmitted by the bite of the tick because coxal fluid is excreted only after feeding. Rafyi et al[593] described a variant of *O parkeri* that was found on the Hastings Reservation in Monterey County, California. The *Borrelia* harbored by this variant differed antigenically from the type strain.

O parkeri can be infested with *B turicatae* but not with *B venezolensis* in the laboratory.[485]

Ornithodoros hermsi and Borrelia hermsii

Ornithodoros hermsi Wheeler, Herms, and Meyer 1935 was described in 1935, together with the *Borrelia* strain carried by it.[730] It was found in the Californian mountains at an altitude between 5000 and 8000 ft, but human cases of borreliosis were discovered also at 3000 ft. It is a disease acquired by persons entering newly opened wooden summer cottages which are frequented (when empty) by wild rodents such as *Tamiasciurus douglasii* and *Eutamias* that often carry this tick in their fur.[358]

B hermsii has been studied extensively by Wheeler.[727-729] It is transmitted with the eggs but less than 2% of them are infested. *O hermsi* does not extrude feces or coxal fluid during feeding. Its bite is infective. When mammalian blood is not available, *O hermsi* may feed on other ticks. Mice and monkeys can be infected with *B hermsii*.[727] Three days after a meal on an infected rodent or man, the celomic cavity becomes invaded, and the central ganglion by the 10th day.[728,729] Longanecker[458] found *O hermsi* in dead trees ("snags") at an altitude of 6000 to 8000 ft. Of 39 batches of *O hermsi*, 18 infected mice, rats, and chipmunks.

Ornithodoros talaje and its Borreliae

Ornithodoros talaje Guérin-Méneville 1848 probably has substrains. It is principally a Central and South American tick, found on the West Coast of the Americas and in Argentina. Bates et al[68] observed *O talaje* in the Arriján area of Panama. Human infections were present there. Rats, mice, and *Macaca mulatta* monkeys could be infected by the bite of *O talaje*. Dunn and Clark[248] described natural infections in marmoset monkeys (*Sangitinus geoffroyi*), *Cebus capucinus,* opossums (*Didelphis marsupialis etensis*), armadillos (*Dasypus novemcinctus fenestratus*), cattle, and in a horse. *O talaje* transfers borreliae rather from animal to animal than from animal to man[182,222] even though it often appears near human habitats.[89] It attaches itself to opossums and other animals that prowl around horses and cattle tied to bush fence posts in which *O talaje* then finds a home. It may also crawl under houses.

Vampire bats and *Triatoma* bugs may acquire this *Borrelia* but do not transmit it to other animals. Some strains of *O talaje* do not bite man.[222,577] The *Borrelia* transmitted by *O talaje* has not yet been named because it was believed that it is identical to that from *O rudis*. Matters became complicated when Calero[137] stated that *B neotropicalis* is a variety of *B recurrentis* carried by *O venezolensis* (synonym: *O rudis*) as well as by *O talaje*. Mazzotti[484,485] found incongruities between the bionomics of *O venezolensis* and *O talaje* on one hand, and *B venezolensis* on the other hand. Davis[225] stated that *O dugesi* is a possible alternate host of *B talaje,* and described *B mazzottii sp.nov.* from *O talaje* from Mexico and Guatemala that transmitted this *Borrelia* regularly, and *O dugesi* weakly and in a fleeting way. *O talaje* from other areas (Panama) did not transmit *B mazzottii,* nor did *O venezolensis, O turicata* from Mexico, and some other ticks. There was no transovarian passage in *O talaje* carrying this *Borrelia*. Guinea pigs and young rabbits were refractory to it. Considering further that *B venezolensis* (synonyms: *B venezuelensis* Brumpt 1924, *B neotropicalis*

Bates and Saint John 1922) is present in *O rudis* Karsch 1880 (synonyms: *O venezuelensis* Brumpt 1921 and *O venezolensis*) in approximately the same area, and that the differentiation of borreliae by immunologic and serologic means is most difficult, one could assume that *O talaje* carried *B mazzottii* and in addition a hitherto unnamed *Borrelia,* the vector being perhaps a subspecies of *O tala-je.* Further investigation of this problem is certainly indicated and, until such studies are carried out, one has to keep an open mind.

Ornithodoros venezolensis and Borrelia venezolensis

The synonyms were discussed under *O talaje,* which is said to be able to carry also *B venezolensis.* Pifano[573] found this tick in Venezuela and in Mérida on the Yucatán (Mexico). A "related" tick was discovered in Yayacuy. The tick acquired the habits of a bedbug, and became domesticated. Rats and mice on which the tick was fed developed borrelemia. The *Borrelia* was neurotropic. No animal reservoir was found. The ticks appeared to feed only on man, as shown by precipitin tests with sera against various animal blood. Osorno Mesa[558] found *O venezolensis* in Santander, Colombia, and suspected that human cases may exist there. Anduze[18] collected *O venezolensis* in the Mérida area of Venezuela at an elevation between 1600 to 5000 feet. The human relapsing fever caused by its bite was severe. León and León[443] found the vector in Esmeraldes, Colombia, and varying clinical pictures of the disease. They suspected birds and wild rodents as the reservoir. Mazzotti[484] believed that *B venezolensis* is the causative agent of relapsing fever in Panama, Colombia, and Venezuela, that *O talaje* and *O venezolensis* carry it, but perhaps that the strains are different.

Other Borreliae

Ornithodoros brasiliensis Argão–Beaurepaire 1923 carries *Borrelia brasiliensis* Davis 1952.[224] This tick has one larval and 4 to 7 nymph stages before the adult emerges. It is able to feed two days after moulting. Davis was the first to rear these ticks in the laboratory. Little information is available about the disease caused by *B brasiliensis* and the ecology of the ticks. It appears, however, that *B brasiliensis* can be transmitted to mice and guinea pigs.

Heisch[345] described *Ornithodoros graingeri,* the vector of *B graingeri* Heisch 1953 in Kenya. It caused disease in man, with slight neurotropism. The illness was mild in rats and mice. Guinea pigs, young rabbits, and monkeys were not susceptible.

Garnham[293] reported a *Borrelia* from a grivet monkey.

Carley and Pope[144] described *Borrelia queenslandica* from *Rattus villosus* in Australia. It caused relapses in mice and rats. Guinea pigs and chickens were not suscep-

tible. This *Borrelia* could be carried in the laboratory in fertilized chick embryos. It was not transmitted by *O gurneyi,* the only *Ornithodoros* species in the area where the organism was isolated.

Further data are not available on these borreliae.

Other Ornithodoros

Ornithodoros coniceps Canestrini 1890 was considered a possible vector of borreliae but Chagin and Diatlov[151] demonstrated that it is not infected in nature. However, about 2% to 3% of these ticks will take up *B persica* when fed on infected animals, and will transmit this *Borrelia* to about 10% of the guinea pigs on which they are fed at a later date. Ovarian transmission has also been observed.

Ornithodoros lahorensis Neumann 1908, which was said to be a vector, was unable to transmit Central Asian borreliae in the hands of Pavlovskii and Kuzima.[567] *O lahorensis* is common in the stone walls of old caravanserais, in cracks of wooden buildings, and in sheep stalls. Perhaps it lives also with rodents in their burrows.[584]

Ornithodoros foleyi Parot 1928 (synonym: *O franchinii*) was considered a vector of *B hispanica* in Lybia where two war-time louse-borne epidemics raged. Colas-Belcour and Vervent[191] were unable to prove that it plays a role in the propagation of relapsing fever.

Ornithodoros savignyi Audouin 1827 has been observed from Timbuktoo to Ceylon. It has never been found to be infected in nature although it takes up *B duttonii* and *B hispanica* in the laboratory.[121] Considerable research has been devoted to this tick which lives outdoors, especially in places where camels, sheep, and other livestock rest. The reason is that it appears in areas where relapsing fever is present but generally accepted tick vectors are scarce, sporadic, or have not yet been found. One of these countries is Somaliland. Moise[497] suspected but did not prove that *O savignyi* is an effective *Borrelia* vector. Kirk[410] could not transmit *B recurrentis* to it in Abyssinia. Walton[710] showed that *O savignyi* is a pure field tick. Lovett[461] in Somaliland found *O moubata* to be a potent vector, but still believed that *O savignyi* may participate in the dissemination of relapsing fever. Anderson[16] pointed out that *O moubata* lives mostly indoors and has much more intimate contact with man so that it must be a much more effective vector.

Ornithodoros canestrini Birula 1845 was investigated as a possible vector of borreliosis by Delpy et al[236] in Iran but neither *B persica* nor *B microti,* the borreliae of Iran, could be transmitted by this tick in animal experiments.

Unusual Vectors

A number of arthropods other than human lice and *Ornithodoros* have been considered as possible vectors of

human relapsing fever. Bedbugs *(Cimex lectularis)* are the first in importance among these.

Rosenholz[611] surveyed the literature and in his own experiments found *B duttonii* in the gut of bedbugs for 5 days after feeding, but borreliae did not find their way into the celomic cavity in all the bugs that were fed. *B duttonii* survived for about 2 months in those that were successfully infested. Chung,[172] studying an outbreak of epidemic relapsing fever in an orphanage in Peiping, stated that bedbugs may acquire *B recurrentis* but the organism vanishes from their blood in one day. Furthermore, Chung and Feng[178] concluded from the results of their experiments that bedbugs, like lice, have to be crushed to transmit the infection. Francis[278] succeeded in transmitting *B turicatae* by *C lectularis*. Bonné[100] stated that while bedbugs and *Melophagus ovinus* can harbor borreliae for about 2 days after feeding, they do not transmit the organisms to mammals. Blanc et al[82] observed considerable differences in the survival time of borreliae in bedbugs according to the microbial strain employed in the experiment. *B hispanica* could be detected for 2 days, *B persica* for 3 to 48, *B duttonii* for 150, and *B merionesi* for more than 200 days after infestation. The virulence of the borreliae did not diminish during their sojourn in the bedbug. Weyer and Moser[726] were able to preserve *B recurrentis* and *B hispanica* in frozen bedbugs.

The South American *Cimex rotundatus* was considered a possible vector of *B venezolensis* by Pino-Pou.[577]

The dog tick, *Rhipicephalus sanguineus,* has been another object of numerous querries. Sergent[637] found this insect, infected, on a dog belonging to a patient with relapsing fever in North Africa. Sergent[639] later reviewed the literature on this subject. Bonné[100] observed infected larvae. When the infective meal took place during the nymph stages, the adult *R sanguineus* carried borreliae for about 3 months.

Members of the tick genus *Argas* are transmitting fowl borreliosis. It is natural that their relationship to human relapsing fever has also been investigated. Bonné[100] was unable to transmit African borreliae by *Argas reflexus.* Harold[337] asserted that *Argas persicus* does not bite man and is, therefore, not a probable vector of human relapsing fever.

The tropical rat mite *(Liponyssus nagayoi)* was studied by Omori.[554] It transmitted *B duttonii* from mouse to mouse for 12 days after the infective blood meal. Its feces and ova remained free from borreliae.

Fränkel[280] attempted to transmit epidemic relapsing fever from man to man by the stable fly *(Stomoxys calcitrans).* This fly, as many other biting insects, is able to harbor live borreliae in its gut for a few days but cannot transfer the organisms to man.

It is possible to paraphrase an axiom by saying that several borreliae may be looking for a vector, and several vectors for feasible hosts. Potential rodent reservoirs without active carriers of borreliae exist in several parts of the world. Huang,[375] for instance, enumerated *Microtus mandarinus, Cricetus triton, C barabensis, Micromys minutus,* and *Apodemus agrarius,* rodents living in the Yang-tse Valley in China, which represent such a potential reservoir. The disease is absent, however, principally because of the lack of an efficient and infected tick vector.

Portals of Entry

Transmission of borreliae takes place most often during the feeding of anthropophilic vectors of human pathogenic *Borrelia.*

During gestation, borreliae seem to be transmitted from the blood of the mother to the fetus,[19] but the organisms are not transmitted through the milk to the offspring.[292,602] Infection may be acquired from the nursing mother, however, through mucous membranes.[73] Menstrual blood also carries *Borrelia.*[116] Skin excoriations as well as intact mucosal membranes may serve as portals of entry.[54,107,364] Chung[174,175] in China, observed that human urine and prostatic fluid may harbor borreliae. He was able to transmit the organisms to susceptible animals in the laboratory.

Laboratory infections have been described as having originated with human clotted blood kept for six days at room temperature,[464] infected monkey blood,[642] blood from the vein of a patient which squirted into the nose of a technician,[429] and in one case, infection occurred in a laboratory worker who accidentally splashed patient's blood into his eyes and in another who was sprayed accidentally with placental blood.[462] A classical example of a laboratory infection is that of an entomologist who was contaminated with the blood of a squirrel he was dissecting.[442] Borreliae may be transmitted also with blood transfusions.[596,716] These are rare exceptions, however, and the classical route is infection by a feeding human louse or *Ornithodoros.*

Attempts to Classify Borreliae According to Response of Experimental Animals

The reader may have experienced considerable difficulties in perusing the preceding pages when attempting to sort out the various hosts or when sifting out the responses of experimental animals in the laboratory to infections with the different *Borrelia* strains. This author has to admit that the study of many of the individual reports quoted in this monograph was not always an easy task, for details of technics, of the mode of infection, the age and condition of the animals including their stock were not always made clear in some writings on this subject. The retesting of many data is still a prerequisite for

the formation of a clearer picture of the parasite-host relationship under laboratory conditions. This can be done by checking available reports by experimenting with accessible *Borrelia* strains and standardized methods. A critical evaluation of the procedures hitherto employed needs to be programmed by keeping in sight two goals: first, the ability of the vector to infect a given animal species. The results of such investigations will help to delineate the animal reservoir, the possible formation of biotopes, and therewith, assist in obtaining information useful from the epidemiologic point of view. Secondly, the question has to be answered whether a *Borrelia* separated from its vector and administered to a laboratory animal will or will not cause signs and symptoms valuable for the laboratory diagnosis of the strain.

Transmission by vectors has been discussed in the foregoing chapters, and will be analyzed again in the portion of this monograph dealing with epidemiology. At this point we are concerned with the susceptibility of laboratory animals, and with the possibility of their use in the classification of *Borrelia.*

Mice have been used for a long time in experimental borreliosis.

They are small, easily maintained, and usually on hand in larger laboratories. There is, however, the question of the route of inoculation, and the age and strain of the mice. Gray,[322] for instance, stated that young mice are more susceptible to *B duttonii.* Kemp et al[406] found that mice are easily infected with North American borreliae. Wolman and Wolman[736] confirmed the susceptibility of mice to *B recurrentis.* Coghill and Gambles[186] were able to produce short-term disease in mice by inoculating them with the blood or brain from patients in whom *B recurrentis* could not be found by other methods in the peripheral circulation. Baltazard and his group[46,59,60,61] emphasized the feeble but very constant susceptibility of mice to *B recurrentis* and other borreliae. Baltazard[47] also demonstrated that the time of borrelemia in mice could be prolonged by splenectomy from the usual 3 days to a longer period. Kroó[425] emphasized that the virulence for mice varies with the strain of the *Borrelia* and with the relapse from which the infective material was collected. Durieux and Boiron,[249] in their studies of relapsing fever in Dakar, used mice as indicators for their survey of the occurrence of borreliae in naturally infected animals, injecting the blood of such animals into laboratory mice.

Geigy and Aeschlimann[300,305] showed that *B duttonii* causes more persistent infection in mice than *B recurrentis.* Sergent[638] reported that mice become ill 24 hours after inoculation with *B hispanica* but recover after a short period.

Young mice were preferred and recommended by Geigy and others[300,305,662,663] for laboratory experiments with borreliae. Guggenheim and Halevi[329] demonstrated that thiamin-deficient mice become more seriously ill than well-nourished mice when infected with borreliae.

Baltazard et al[46,59] found the response of adult rats to *B recurrentis* somewhat variable even after splenectomy, but the borreliae were more pathogenic than they were for mice. Newborn rats became consistently infected, and the borreliae circulated longer in their blood than in the blood of mice according to these investigators[48,99] who also preferred rats to mice because of their size and the ease with which they can be handled. Rats usually survive the infection, even though they may appear ill. Geigy and Aeschlimann[305] observed that *B duttonii is* pathogenic but somewhat less for rats than for mice. Kalajew[393] found that splenectomized rats harbor in their circulation enormous numbers of borreliae with a long period of survival of these organisms in the brain. Rats are highly susceptible to the American strains of *Borrelia.*[406,407]

Rabbits appear more difficult to infect. Nicolle and Blaizot[532] had to inject large numbers of *B recurrentis* intravenously to observe borrelemia lasting 2 days. Sergent,[638] using *B hispanica,* found only few organisms and these only for a short time in the rabbit. Geigy and Aeschlimann[305] recorded rabbits as not sensitive to *B duttonii.* Greiner[324] succeeded in infecting young rabbits with *B hispanica.* Baltazard et al[61] were able to produce fatal infection in newborn rabbits, principally after animal passage.

Guinea pigs have developed into a favorite tool for differentiating borreliae. Greiner[324] found them refractory to *B recurrentis.* Wolman and Wolman[736] observed, however, that guinea pigs could be infected with the Ethiopian strain of *B recurrentis.* Baltazard[46] pointed out that these rodents are only exceptionally susceptible to *B recurrentis.* Coghill and Gambles[186] found that *B recurrentis* causes only latent infection in guinea pigs, that *B hispanica* always produces patent infection, and that these animals are refractory to South American borreliae. Kervran[409] observed elevated temperature in guinea pigs after injecting infected blood containing *B duttonii.* Newborn guinea pigs may be more susceptible to *B duttonii.*[304] Sergent[638] produced a disease in these animals with *B hispanica* starting with an acute attack, then becoming chronic. Colas-Belcour and Vervent[191] made similar observations. Davis[219] found guinea pigs susceptible to all North American strains of *Borrelia.*

Baltazard et al[62] suggested the use of guinea pigs in the differential diagnosis of borreliae because they are highly susceptible to *B hispanica, B persica,* and, principally young individuals, to North American strains but not to *B latyschewyii,* the crocidurae subgroup, and in most instances not to *B recurrentis.* Adults, however, were refractory also to *B duttonii* in his experience.

Different species of monkeys and apes have been found

to be susceptible to various species of borreliae. Nicolle and Blaizot[532] produced transient infections in hooded monkeys with all strains of *B recurrentis*. *Macaca sp* was particularly susceptible to the Tripoli strain of relapsing fever. LeGac[439] and Baltazard[46] also observed that *Macaca* and *Cercopithecus* monkeys could be infected with ease. A mild disease resulted. *Cynocephalus* was refractory to his *B recurrentis* strain. *B duttonii* may cause fatal infection in monkeys.[303,304] Rhesus monkeys have been found susceptible to North American *Borrelia* strains, also to South American borreliae.[68] *M inuus* became infected with *B hispanica*[99,638] but minimal pathogenicity of the crocidurae subgroup was observed in *Cynocephalus*. We have found patas (*Erythrocebus patas*) mildly susceptible to North American borreliae.[268] The incubation period varies according to the mode of inoculation.

Dogs have been used by Sergent[638] in the study of *B hispanica*. Young animals were easily infected.

Chickens were recommended for experimental purposes by Kervran,[409] who transmitted *B duttonii* to them.

Hamsters have been little used in *Borrelia* experiments. Chen and Anderson[159] found them susceptible to *B hermsii*. We were able to transmit *B turicatae* and *B parkerii* to them. Splenectomized hamsters infected with *B recurrentis* were studied by Chen et al,[160] who saw mild disease.

Cotton rats (*Sigmodon hispidus*) were found by Varma[701] to be more susceptible to *B turicatae* infection than were white mice.

The possibility of using the European hedgehog (*Erinaceus europaeus*) as an experimental animal was studied by Lapierrre et al.[435] The hedgehog was resistant to *B duttonii,* acquired an inapparent infection when infected with borreliae of the crocidurae subgroup, was susceptible to *B hispanica,* and became seriously ill after infection with *B persica*. However, the response was not always clear-cut and varied with the strains of the respective species.

Some animal experiments have been carried out by unorthodox routes of inoculation.

The infection of white rats by the transnasal route, using *B hispanica,* was successfully accomplished by Nájera Angulo.[514] Joyeaux and Sautet[389] infected rats by feeding them with brains of other rats infected with *B duttonii*. Conjunctival and peroral infection of squirrels with *B recurrentis* was accomplished by Chung.[172] Blanc et al[83] produced *Borrelia*-keratitis in the rabbit eye. *B hispanica* caused a lesion resembling syphilitic keratitis, whereas *B duttonii* and *B merionesi* evoked different pathologic pictures.

Chorine and Crogue[168] quantitated *B hispanica* in guinea pigs. The multiplication of borreliae in experimental animals was also studied by Eidmann et al[252] and

Baltazard et al.[57]

Reinfection of susceptible animals is possible after several months.[533,546]

Summaries of borreliae in laboratory animals may be presented as follows. Nicholle and Anderson[526] stated that:

1. *B duttonii* and its relatives are virulent for mice and rats, hardly at all for guinea pigs.

2. The *B hispanica* group is equally pathogenic for mice, rats, and guinea pigs.

3. Small-rodent borreliae can infect mice but rats and guinea pigs are not sensitive. Members of this *Borrelia* group are mildly harmful or nonpathogenic for man.

Geigy[303] summarized:

1. *B recurrentis* causes infection in monkeys, usually after 2 to 4 days incubation, with borrelemia lasting about 4 days. Adult mice and rats are not very susceptible, developing borrelemia with various intensity. There is no residual brain damage in the infected animals.

2. *B duttonii is* strongly pathogenic for guinea pigs, monkeys, rats, and vervets.

3. *B hispanica* and *B turicatae* may cause disease in guinea pigs, but some are refractory. Monkeys, rabbits, rats, and mice can be infected while young.

4. *B venezolensis* infects rats and mice but as a rule not rabbits and guinea pigs.

We would like to emphasize that the severe response of monkeys to *B duttonii* and that of guinea pigs to *B persica* and *B hispanica* are valuable laboratory aids in differentiating tick-borne Old World borreliae.

Perhaps more extensive studies are needed on animals that harbor certain borreliae in nature or become occasionally infected with them. After standardizing the route of infection, the age of the animals used, and the infective dosage, the differentiation of *Borrelia* strains may become an easier task in the laboratory.

The Interference Phenomenon

Trautmann[689] is said to have been the first to experiment with *Trypanosoma* and *Borrelia* on the same animals in 1907. He noted that rabbit serum against *B duttonii* immobilized but did not agglutinate *T brucei* and vice versa. Daels[214] confirmed these observations. Vassiliadis and Jadin[702] found that *B hispanica* slows down *T rhodesiense* infections but to a lesser degree than *B duttonii* mitigates the disease caused by *T pecaudi*. Rubinstein and Kapusto[615] believed that a new, symbiotic "race" of *Borrelia* may develop in mice in mixed infections. Kawamura[400] reported that the injection of *B duttonii* and *B hispanica* simultaneously causes prolonged infection in mice but that the administration of *B hispanica* or *B crocidurae* together with *T brucei* prolonged the life of the mouse from 3 or 4 days to about 22 days.

Vincent[707] observed that when *T somaliense* and *Borrelia* were injected simultaneously, the incubation period was the same as when both organisms were administered separately, namely, 3 days for the *Borrelia,* and 2 to 5 for *Trypanosoma.* The *Trypanosoma,* however, multiplied slowly and the mice did not die in 5 to 9 days as usual after injection with *T somaliense.* The number of typanosomes increased, and began to appear in the blood periodically. Then, *T somaliense* started to multiply and killed the mice in 30 to 40 days. Vincent believed that the reticuloendothelial system (RES) played a significant role in these variations of suppression.

Carminati[146] experimented with *B duttonii* and *T brucei, T gambiense,* and *T equiperdum* in mice. Interference between the *Borrelia* and the *Trypanosoma* strains was observed only while numerous borreliae circulated in the blood. After their disappearance, the trypanosomiasis ran its usual course. The sera of animals treated with *B duttonii* alone did not show antitrypanosomal antibodies. Lapierre et al[434] and Larivière et al[436] extended their experimental work to *B crocidurae;* Lapierre et al[433] to several strains of *B duttonii, B hispanica,* and *T brucei.* Gaillard et al[286] studied also *T cruzi.*

All investigators found variations in the interference phenomenon according to the strain employed but Gaillard et al[287] felt that this phenomenon could be utilized in the differential diagnosis of borreliae. Mice should be infected with the unknown strain. and *T brucei* injected at the height of the borrelemia. If the mice survive for a long time (2 or 3 weeks), the unknown strain may be *B duttonii* or a member of the crocidurae subgroup. If the mice die within one week, the *Borrelia is B hispanica* or *B persica. B turicatae* gives variable results.

The interference phenomenon may be due to the antigenic and biological resemblance of borreliae and trypanosomes which has been pointed out repeatedly.[264,265]

Borreliae do not interfere, however, with *Spirillum minus* and *Leptospira* infections,[262,465] malaria,[640a] *Coxiella (Rickettsia) burneti,*[81] or coxsackie B[447] infections. Borreliae show certain crossreactions with treponemas and *Proteus OX* strains. This will be reviewed together with the evaluation of the importance of such responses for serologic tests in the chapter on Laboratory Diagnosis.

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Journal of Spirochetal and Tick-borne Diseases

Dedicated to science and art in spirochetal and tick-borne diseases

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