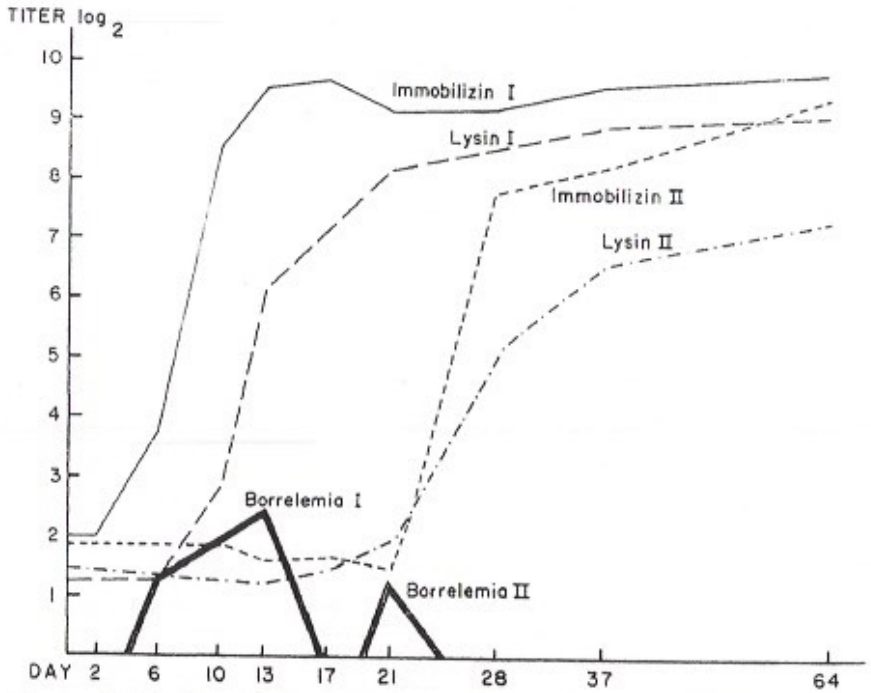

FIGURE 4. Serologic data, patas monkey *(Erythrocebus patas)* infected with *Borrelia parkerii*.

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). Azur-eosine 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 1 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 re-fixed 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° 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 Dressler (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° to 2° C but were killed in 30 minutes at 50°. 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° to 15° C for 7, and at 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. Temperatures between —15° 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° 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 7½ 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° 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 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 co-workers (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

TABLE 2
SIZE OF SOME ARTHROPODS CARRYING BORRELIAE AND THEIR NICKNAMES+

Scientific Name	*Male**	*Female*	*Nickname*
Pediculus humanus corporis	2.5-3.3 x 0.8-1.1	3.2-3.6 x 1.1-1.4	
Pediculus humanus capitis	1.6-2.1 x 0.6-0.8	2.4-2.8 x 0.9-1.1	
Ornithodoros moubata	4.2-5.8 x 3.7-4.2	7.8-12.8 x 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 x 1.8-2.5	4.2-6.8 x 2.4-4.1	
Ornithodoros tholozani	3.5-6.2 x 2.8-5.2	7.6-9.1 6.8-7.4	
Ornithodoros rudis	3.3-4.2 x 2.4-3.3	4.8-6.4 x 2.8-4.2	Cuescas, Mordijini (Venezuela) Talajas (Colombia)
Ornithodoros talaje	4.8-6.2 x 3.4-5.2	5.3-7.4 x 4.7-6.2	
Ornithodoros turicata	2.7-4.4 x 2.3-2.9	4.6-6.9 x 3.3-4.3	Pajaroello (Mexico)
Argas persicus	4.0-5.5 x 2.6-3.3	5.0-10.1 2.4-7.5	

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

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*, i.e., 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 cloth-

FIGURE 5. Head of louse, *Pediculus humanus*.

FIGURE 6. Claws of human louse, *Pediculus humanus*.

FIGURE 7. Male louse, *Pediculus humanus (corporis).*

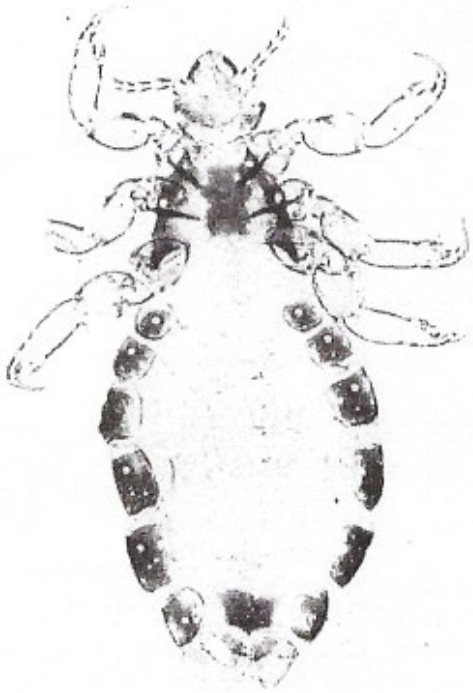

FIGURE 8. Human louse, *Pediculus humanus (corporis),* female. Slide preparation.

ing and hatch at 28° 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 meal 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 4,407 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 cork-screw-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- 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 (O.F.) 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 co-workers (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, cork-screw 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 adaptation. 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 phyllum 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 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.

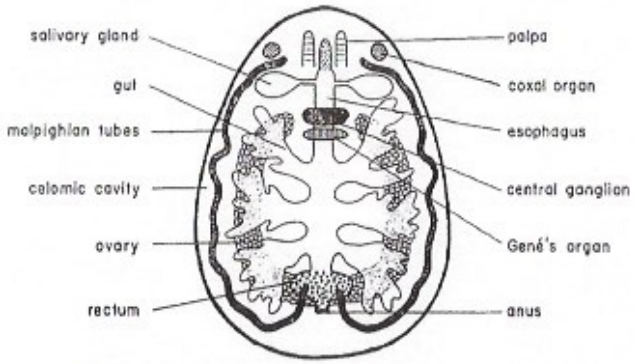

FIGURE 9. Schematic section through an *Ornithodoros*.

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. 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 cork-screw-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 U.S.S.R., 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 follows. 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 subgroup 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. cholodovskyi* Pavlovsky 1930 lives in Turkestan; *O. lahorensis* Neumann 1918 in

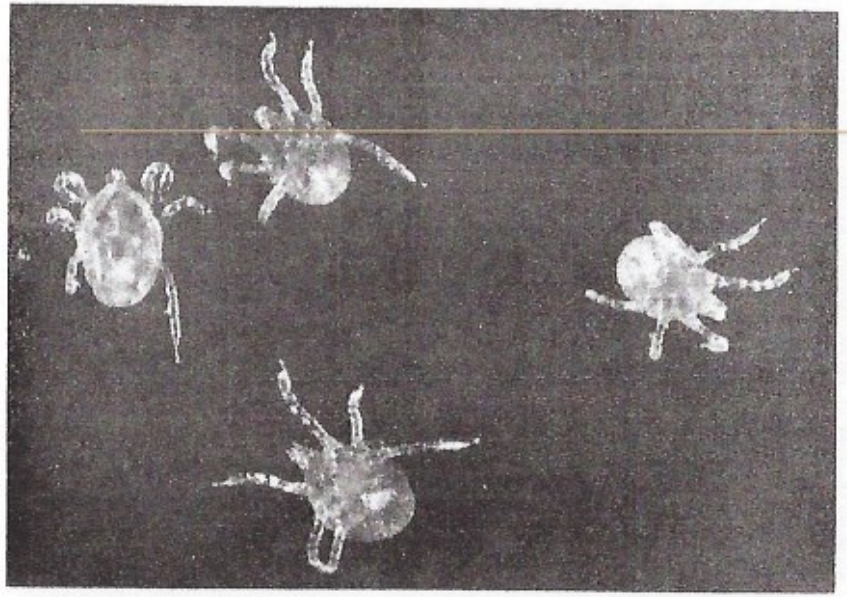

FIGURE 10. *Ornithodoros tartakovskyi,* nymphs. Photograph by Dr. T. C. Orihel.

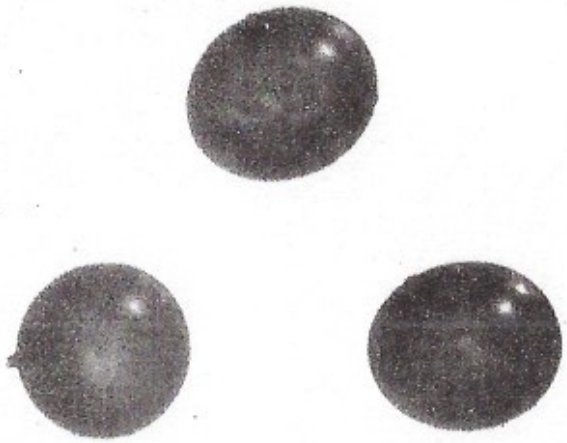

FIGURE 11. *Ornithodoros tartakovskyi* eggs.

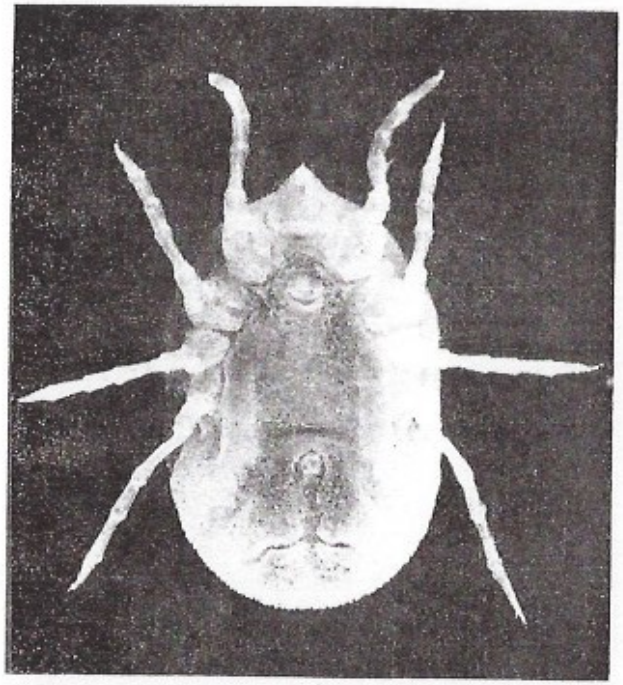

FIGURE 12. *Ornithodoros tartakovskyi,* adult femals, ventral view. Photograph by Dr. T. C. Orihel.

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 re-

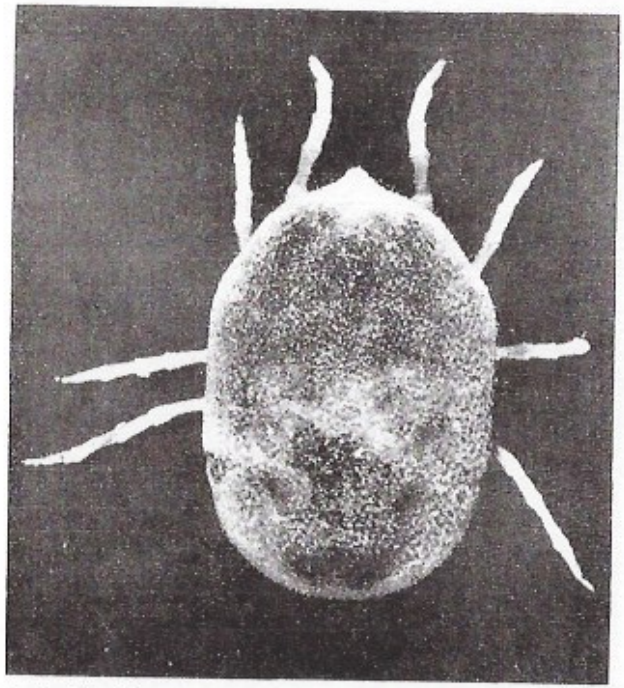

FIGURE 13. *Ornithodoros tartakovskyi,* adult female, dorsal view. Photograph by Dr. T. C. Orihel.