BIOLOGICAL RELATIONSHIPS BETWEEN LICE (ANOPLURA) AND MICROBIAL AGENTS

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INTRODUCTION

Normally the bodies of sucking lice (Anoplura) are free from pathogenic microorganisms. Sterility of the intestinal contents is one of the important factors determining the optimal conditions for life of lice, which can even rid themselves of microbial agents—at least to a certain degree. Symbiotes are the only permanent and obligatory residents of the bodies of lice. They furnish nutritive substances which are indispensable for the growth and reproduction of their hosts [Aschner (3); Puchta (61)].

On the other hand, lice are known to be vectors of spirochetes and rickettsiae; epidemic typhus, trench fever, and relapsing fever are "louse-borne diseases." Not until relapsing fever and certain rickettsial diseases were investigated, were lice and their role as transmitters of disease agents studied more thoroughly [Buxton (8); Hase (28)]. Today we have a fair knowledge of the relationships between the sucking lice and some of the more important pathogenic agents. We know hardly anything, however, concerning those internal biochemical processes which govern the association of lice and microbial agents. For practical reasons, the main object of investigation has been the body louse, Pediculus humanus humanus Linnaeus. Our knowledge regarding the same relations between animal lice and microbial agents are much more incomplete.

While fundamental observations on the role of lice as vectors of diseases were made years ago, recent investigations have broadened our knowledge. Spirochetes and rickettsiae are probably the only true parasites of sucking lice. Hence we will deal mainly with these parasites in this paper; only the more significant observations and experiments that pertain to the behavior of other blood parasites borne by lice, and their transmission, will be reported. Here the relationships between host and parasite are either accidental and short-termed or have not been sufficiently explained as yet. Since many of the observations made are based upon laboratory experiments, the techniques that have been applied have a bearing on the results and must, therefore, be briefly discussed.

NOTES ON RESEARCH METHODS

For experimental work, the rearing of body lice under laboratory conditions is essential. For detailed descriptions of breeding methods see Nauck & Weyer (50); Reichenow, Vogel & Weyer (63); Sikora (67); and Smith & Eddy (69). True head lice, Pediculus humanus capitis DeGeer, and crab lice,

1 The survey of the literature pertaining to this review was concluded in April, 1959.
Phthirus pubis (Linnaeus), can experimentally be maintained on man for a limited time only. Animal lice are reared on their natural hosts. Haemotopinus suis (Linnaeus), normally living on pigs, will survive for a few days if fed on human blood [Weyer (85)]. The lice are kept in wooden or metal cages, one side of which consists of gauge to permit suction, and under as sterile conditions as feasible. Normal reproduction—yielding an excessive number of lice—can be achieved by maintaining the lice at 30°C, and feeding them for 30 min. daily. Other methods were developed in order to avoid the somewhat difficult procedure of feeding lice on man; for example, animal or artificial membranes were used, the nutrient being defibrinated blood [Fuller, Murray & Snyder (23); Hadden (27)]. Here, however, lice can be maintained only for a limited time while their propagation rate is low.

A considerable improvement of rearing methods was achieved when strains of body lice could be experimentally selected as to develop normally if fed on rabbits [Culpepper (16); Pashenichnov & Noskova (60)]. Contamination of the colony by bacterial infections, however, occurred more frequently than after feeding the lice on man. Feeding on man will also considerably facilitate simultaneous experimental work with a large number of infected lice.

To investigate the relationships between lice and the microbial agents they harbor, lice gathered from sick persons or animals under natural conditions can be directly or indirectly examined for microorganisms, although better information will usually be obtained by experimental inoculation of lice. Normally lice take up microorganisms only when sucking; invasion of the stomach via the rectum—proceeding from moist feces, for example—will occur only if the individual louse is old or weakened by sickness. Lice can be inoculated in the natural way by feeding them on infected hosts. Body lice do not necessarily require human blood; for a single time, at least, they may be fed on monkeys, guinea pigs, rats, mice, or rabbits. Mortality resulting from feeding lice on a nonspecific host can be decreased by holding the lice at 37°C for 1 or 2 hr. immediately after feeding and offering them human blood soon after [Cabasso (9)]. For experiments with strains adapted to rabbits, the lice may be fed on rabbits that have previously received an intravenous injection. As proved by Snyder & Wheeler (70), rickettsiae will remain in the blood of rabbits—which were inoculated by use of a yolk-sac suspension, for instance—for at least three days. Small amounts of microbial agents may be intradermally injected (bleb technique), thus permitting lice to take up the agents when sucking from the bleb area. This method is particularly useful for infecting the youngest larval stages of lice. Inoculation is greatly facilitated if the lice are fed through membranes, since a larger amount and a known concentration of microbial agents may be added to the blood used as nutrient.

The study of the relationships existing between lice and microbial agents was considerably advanced by the discovery that experimental inoculation of lice by means of very thin glass capillaries is possible [Welg (79)]. This method has proved useful until today, as it is both simple and exact [Krydski & Radkowski (34); Weyer (80)]. By application of this technique, not only blood but also suspensions of organs and concentrated doses of agents may be inoculated into the stomach of the louse. The behavior of the microorganisms and the reactions of the louse will be the same if the agents invade the louse via the rectum or in the natural way, i.e., via the esophagus. Furthermore, lice may be intracoelomically inoculated [Weyer (86)]; for this female specimens are suited best, because inoculation can be made via their genital openings. Both inoculation methods are used not only for research work but also for maintenance of pathogenic strains in lice.

Non-specific or insufficiently explained relationships between Lice and Microbial Agents

Lice, which are stationary blood-sucking ectoparasites, have frequently been suspected of transmitting other microbial agents than spirochetes and rickettsiae because their infestation of man is often accompanied by various other diseases. Hence, some observations and experiments will be discussed which were devised to demonstrate whether certain agents will survive and propagate in lice and whether lice must be considered transmitters of these agents.

Suspected or tested microorganisms include viruses, bartonellae, bacteria, protozoa, and filariae. Negative results were obtained by experiments with filariae; certain species of Plasmodium, Leishmania, Anaplasmata, and Leptotrichia; Trypanosoma ruissi (Steel); Tseeplasma gondii Nicolle & Manceaux, and the agent of the swamp fever. The number and methods of the experiments concerned, however, do not always permit final conclusions. Besides body lice, various animal lice were investigated. It is worth mentioning that T. gondii—injected intracoelomically—not only lived up to 13 days in the hemolymph of the louse, but also remained virulent and capable of reproduction, though no multiplication took place in the hemolymph itself [Weyer (82)]. Positive results were gained from experiments with the viruses causing trachoma (Cahnod, Natal & Lovekitch (15)), lymphocytic choriomeningitis (Milker (40)), rabbit myxomatosis (Button (7)), and yellow fever (Philip (57)). But all these experiments proved only that these agents may be ingested by lice when sucking and that they will survive for a certain time in the body of the arthropod.

We are better acquainted with the relationships existing between lice and certain bacteria. Alverdes & Rieling (2) administered various bacteria to body lice either by feeding the latter on rabbits or inoculating them via the rectum. Both methods yielded the same results: certain large gram-positive cocci, which are occasionally found in or in the body of lice, remained in the stomach lumen of lice for a longer time—their reproduction rate being low—without harming their hosts. Influenza bacilli, pneumococci of Type I, and Bacillus cereus var. myxoides (Flügge) could no longer be demonstrated after a maximal period of 48 hr. On the other hand, a true infection was gained
after the lice had been inoculated with typhoid, paratyphoid, and cholera bacilli. The bacteria invaded the stomach cells and multiplied intensively. The infection caused a rapid destruction of the stomach cells, which had become distended, and led to a detachment of the mucous membrane from which the lice died. The bacteria were voided with the feces.

Similar experiments were carried out by Milner, Jeilina & Smith (39). The lice ingested blood containing bacteria either through membranes or by feeding on infected rabbits. Principal investigations were made on Salmonella enteritidis (Gaertner); parallel tests were carried out on Salmonella typhimurium (Loeffler), Salmonella pullorum (Rettger), Salmonella paratyphi A (Kaye) and Shigella dysenteriae (Shiga). S. enteritidis multiplied in lice rapidly, the maximum number being reached after 6 to 8 hr. Ninety per cent of the lice died on account of the infection within 48 hr. Salmonella survived in dead lice and feces of lice for more than 1 yr. and could be transmitted to rabbits by means of suspensions of triturated lice and feces, Krysko, Kucha & Rech (33) transferred suspensions of Proteus cultures (OX 19) to lice by Weigl's method. The majority of lice died within 48 hr. as a result of degeneration of the mucous membrane of the stomach caused by a toxic effect. Only a few lice succeeded in overcoming the infection. The bacteria remained in the lumen of the stomach and did not attack the stomach cells.

Lice have also been suspected as carriers of leprosy and plague bacilli, although investigations of this matter are rare and incomplete. In several cases acid-fast bacilli were found in lice infesting people who suffered from leprosy, but it still remains to be proved in some cases whether these acid-fast bacilli were really leprosy bacilli. Marchoux & Chorine (37) were able to transfer rat leprosy by inoculation of healthy rats with suspensions of lice collected from infected rats. By the same method plague bacilli could be transmitted to rodents by body and head lice [Long (36); de Raadt (62); Swellengrebel & Otten (76)]. In several instances, plague bacilli could be demonstrated in various rodent lice [Link (35) and others]. Observations by Blanc & Bataillard (3), who claim that pig lice are capable of transmitting plague bacilli from infected to healthy guinea pigs by biting, have not been confirmed. The same authors found that lice harboring plague organisms acquired this infection from humans during the septicaemia, and that the agent may remain in feces for at least 7 days in lice and 9 days in the feces of lice.

In several cases, transmission of tularemia to mice, guinea pigs, and rabbits by various rodent lice was achieved [Francis & Lake (19); Girard (25); Parker (56) and others]. These findings were experimentally supported by Price (59, 59). The lice ingested rabbit blood which contained Pasteurella tularensis (McCoy & Chapman) through a membrane. Afterward the lice and their feces were preserved at various temperatures and relative humidities to test the longevity of the bacteria, which was mainly affected by the temperature. The bacteria remained alive in the feces for a shorter span than in the lice if humidity was very high, for a longer time than in lice if humidity was low. At 29°C and a relative humidity of 0 per cent, for example, the bacteria survived for only 0.5 days on blotting paper, for 17.0 days in dead lice, for

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11.5 days in fasting lice, while they were still alive after 22.5 days in house feces, after 26 days at 20°C, and even up to 43 days at 4°C. Thus feces of lice proved to be a valuable medium for preservation of bacteria. Generally, all lice harbored bacteria after feeding on infected rabbits. The number of bacilli harbored by lice was dependent on the amount of bacteria ingested. The number of lice containing no bacteria increased with the time elapsing since the infectious blood meal. In some lice, however, bacterial reproduction was high, bacteria of unchanged virulence being voided with the feces. Several lice harbored bacteria up to 35 days. Out of 13 healthy rabbits, on which infected lice were fed, 10 died of tularemia 5 to 16 days after the first feeding of infected lice.

There seems to exist certain relationships between lice and bartonellae. Cannon & McClelland (10), Crystal (14a, 14b), Elliot & Ford (18), Mayer (38), and Timmermann (77) were able to transmit Haemobartonella muris (Mayer) to rats using rat lice [Polyplax spinulosa (Burmeister)] as vectors. For this purpose, the lice were transferred from infected to healthy rats. Furthermore, Elliot (17) showed that Eperythrozoon cayeyense Schilling of mice can be transmitted by Polyplax serrata (Burmeister). There had been no conclusive evidence of reproduction of these agents in lice. Crystal (14a, 14b), however, found that the infectiousness of the haemobartonellae is enhanced in lice within four to seven days after feeding on infected rats. In most experiments the mode of transmission had remained unknown. According to Crystal, transmission occurs normally by the rat crushing infected lice and inoculating itself by scratching and biting. Transmission may also occur directly by bartonellae remaining on its mouthparts if lice, after having fed on an infected rat, proceed immediately to feed on a second rat. Lice may also have been suspected of transmitting verruca peruana in Colombia [Weinman (81)]. This assumption could not be proved by experiments with Bartonella bacilliformis (Strong e110) [Wigand & Weyer (93)]. In the course of these experiments, body lice were inoculated either rectally or in the natural way by feeding them on infected monkeys. The bartonellae were never seen to establish themselves or multiply in lice. It is worth mentioning, however, that the number of bartonellae greatly increased in the hemolymph of lice, if they had been intracoelomically inoculated, and could later be cultivated on culture media.

Most results of the investigations previously mentioned indicate only that microbial agents, present in the blood of mammals, will be ingested by lice, where they may remain virulent for some time in the stomach, and may be—at least to some extent—voided with the feces. There is nothing peculiar about this, and it is quite natural, too, that such lice may become a source of infection, either by being crushed on the skin or by means of their feces. Here lice must be regarded as only mechanical transmitters, similar to other blood-sucking arthropods or house flies, though of little practical importance because of their stationary mode of life. There is no evidence of intimate biological relationships between these blood parasites and lice.

Differences in reaction of lice to infection by these blood parasites may
arise primarily from properties of the agents themselves. Some of these will die in the stomach, while the majority will probably be voided with the feces. Frequent blood meals and active digestion cause regular drainings and cleanings of the alimentary canal, which may be effective, even in cases of severe infections, after several days, as is the case with P. tularensis. Some agents, however, particularly if present in great numbers, are capable of remaining alive in lice and multiplying either extracellularly in the lumen of the stomach or intracellularly in the cells of the stomach wall. The reasons for this difference in behavior are not known. In some cases, e.g. with Proteus and Salmonella, growth of the agents will be attended by a severe toxic effect. Even if the invasion of the agent must be considered a true infection, the quick lethal effect on lice makes a biological relationship appear doubtful. Since P. tularensis is capable of reproduction in the alimentary canal of lice and will be voided with the feces as a virulent agent even after weeks, it may be that this relationship represents the first stage of a biological adaptation between both organisms.

The practical consequences of these facts are probably insignificant. Infection of lice on man requires a severe septicaemia, which will hardly be sufficient in cases of tularemia and salmonellosis. If louse infestation is extensive, lice—in association with epidemic typhus and relapsing fever—may play a role in spreading Salmonella [Müller, Jellison & Smith (39)]. Here, as in the case of tularemia, the feces containing the microbial agents must be considered the actual danger. There is, of course, no evidence of transmission by biting or by transovarial transfer [Price (59)].

Relationships Between Lice and Rickettsias

Body lice are the natural hosts and vectors for Rickettsia prowazekii da Rocha Lima, the agent causing epidemic typhus, and for Rickettsia quintana Schminke, causing trench fever. Larval and adult stages are equally susceptible to the rickettsias. After lice had been suspected of transmitting classic epidemic typhus rickettsias for many years, the agent and its behavior in lice were described in detail for the first time by da Rocha Lima (65). Munk & da Rocha Lima (48) reported also on the role of lice as transmitters of R. quintana and on the behavior of this agent in lice.

R. quintana occupies an exceptional position among the rickettsias which are pathogenic for man [Mooser & Weyer (45); Weyer (87)]. These rickettsias do not invade the insect's stomach cells. Their reproduction is exclusively extracellular, either on the epithelial cells, where they frequently form a rodlke fringe, or in the lumen of the stomach. Viability and longevity of the lice are not affected, even if the rickettsias grow rapidly. Once infected, lice will harbor the agents for life. As the rickettsias remain confined to the stomach and will leave their hosts only by means of the feces, lice feces are the only source of infection for man. The assumption that, besides R. quintana, other rickettsias less pathogenic or apathogenic for man will grow in lice extracellularly [Herzig (32); Mosing (47)] cannot be maintained any longer. We have good reason to believe that Rickettsia prowazekii Munk & da Rocha are identical with R. quintana [Codd-}

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Lima and Rickettsia weigl Mosing are identical with R. quintana [Codd-}

Another species of rickettsiae has been observed several times in lice which had been reared under laboratory conditions. This species, whose origin could not be detected, was named Rickettsia rochsiimeri Weigl [Sparrer (71); Weigl (80)]. The striking features of this species, according to the available reports, are its ability to multiply in the louse stomach both extra- and intracellularly without severely harming its host and its high infectiousness for lice. These rickettsiae, which are very resistant, will even invade healthy lice after short contact with the dried feces. Thus the occurrence of this species in a louse colony will lead to an infection of all lice within a few days. At present, laboratory strains of this species do not exist.

Although lice are considered natural vectors for R. prowazekii only, Rickettsia typhi (Wolbach & Todd) is the agent causing murine typhus, behaves principally in the same way as R. prowazekii when harbored by lice. Both species grow intracellularly. The rickettsiae which have reached the stomach invade the cells of the mucous membrane, where they propagate so intensively during a few days that the entire cell plasm will be filled with rickettsiae. Accordingly the cells of the mucous membrane will become detached until they cannot resist the pressure any more and will burst, shedding their contents into the lumen of the alimentary canal. From here the rickettsiae will be carried away by the feces, and then can invade humans via skin injuries or through mucous membranes. Most lice die 8 to 12 days following ingestion of the rickettsiae. This is a distinctive property of lice as compared to fleas, whose longevity is not affected by the rickettsial infection. In lice, the rickettsiae do not invade salivary glands or eggs. Under natural conditions they will reach the stomach only when lice suck blood; an infection of lice by contact with feces which contain rickettsiae does not take place.

Using the membrane technique [Fuller, Murray & Snyder (23)], Fuller (20) made a comparative study on the susceptibility of lice and cotton rats for detecting very small amounts of rickettsiae. Both methods proved to be of equal value. Furthermore, he fed lice through membranes on suspensions which contained rickettsiae and after this on rabbits [Fuller (21)]. Applying this method, he was able to maintain a strain of R. typhi through 19 passages for 95 days continuously in lice, and a strain of R. prowazekii through 5 passages for 79 days. R. typhi also multiplied if the lice were fed on human serum. The effect of a R. prowazekii infection on the longevity of lice was studied by Fuller (22), too. On an average the infected lice remained alive for 15.4 days at 25.6°C. (control lice 26.3 days), for 7.4 days at 31.9°C. (control lice 12.5 days), for only 2.4 days at 35.8°C. (control lice 2.4 days also). The dose of the
infectious material, which was increased to one thousand times the original amount, however, affected the longevity of the lice only slightly.

Pathogenicity of *Rickettsia* for lice arises from the fact that the stomach epithelium loses its functional properties. The main damage is done by a mechanical effect: the cytoplasm—the nucleus is never invaded—is consumed and destroyed by the intensively growing *Rickettsia*. The stomach cells may also be harmed, in cases of slight *Rickettsia* infections, by a toxic effect. This implies that pathogenicity is not a simple function of the number of microbial agents. The result is liquefaction and vacuolization of the cytoplasm at its basis and, in extreme cases, detachment of single cells or larger cell shreds. The destroyed cells cannot be replaced, since lice have no regeneration crypts like other insects. Hence the stomach epithelium of lice will be more or less injured if they suffer from a *rickettsial* infection. This state is externally recognizable by the reddish color of the lice, resulting from hemoglobin that was ingested when feeding and which now enters the hemolymph through the damaged stomach walls. Such "red lice" will survive for a few hours only. During this period *Rickettsia* may leave the stomach cells and invade the hemolymph, where they may continue to grow.

The normal development of *Rickettsia* in lice shows variations that are attributed partly to certain properties of the strains, partly to periodical fluctuations of virulence in the same strain. These variations become most evident when the growth rate of *Rickettsia* and their pathogenicity for lice are studied. Also, because of these variations, the first *Rickettsia* may be found in smear preparations of the stomach on the second day or not before the eighth day after inoculation, and the infection may prove lethal as early as the second day, or not at all. There are also certain properties of the lice which have a bearing on the infection, regulating it to some extent [Weyer (86)]. Although the same doses of infectious material were used, *Rickettsia* were rapidly growing in some lice of a batch, while they multiplied more slowly in others. There were also lice which appeared to be healthy, their stomach cells being only sporadically infected. Sometimes lice, whose entire mucous membrane had been invaded by the microbial agents, had a normal life span. Other experimental results can be explained only by suggesting that in some lice *rickettsial* growth is not only temporarily or permanently retarded, but that *Rickettsia* which have been multiplying after invasion of the cells are destroyed. Some observations [Reichmuth (64)] seem to imply that resistance to *Rickettsia* is related to certain geographical races which may occur within one population, such as in dark-pigmented lice. However, the evidence which is available cannot be considered conclusive yet.

The number of lice which will become positive after feeding on a patient suffering from epidemic typhus depends on the extent of *rickettsial*iaemia. Usually this is sufficient for infection only during the first two weeks of illness, but lice may also become infected while feeding on people suffering from Brill-Zinsser disease, in which case *rickettsial*iaemia is low [Murray & Snyder (49); Weyer & Hornbostel (91)]. With trench fever, in contrast to the classic epidemic typhus, a *rickettsial*iaemia lasting for several weeks or even months—and sufficiently severe to allow infection of lice—is the rule.

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Besides *R. quinella*, *R. prowazekii*, and *R. typhi*, there are also other species of *Rickettsia* capable of reproduction in lice. Species or strains that have been successfully transferred to the mucous membrane of the human stomach, where multiplication took place, either by feeding lice on infected mice and guinea pigs or by rectal inoculation are: *Rickettsia rickettsii* (Wolbach) [strain Michoscan from Mexico, strain Bitterroot from U.S.A., one strain from Brazil]; *Rickettsia conorii* Brumpt (one strain from North Africa, one strain from Kenya, one strain from South Africa; two strains of Siberian tick-bite fever *Dermacentor variabilis* Zadorovsky); *Rickettsia akari* Huelner et al. (strain Kaplan from North America, one strain from Russia); *Rickettsia australis* Philip and one strain of *Rickettsia burnsii* (Dorrick) [Weyer (85, 86, 88, 90)]. Virulent *Rickettsia* were voided with the feces of the lice. Their number, however, was rather small. Sometimes—at late stages of the infection—the hemolymph, too, was invaded and there the *rickettsiae* continued to multiply. *C. burnetii* was capable of infecting other organs from the hemolymph. The strains could be easily maintained through passages in lice by use of suspensions of infected stomachs as an inoculum; without that the strains would change their properties. In several cases, maintenance of these strains proved to be easier than rearing *R. prowazekii*. Regarding their pathogenicity for lice, the species and strains which were tested showed significant differences. Apparently pathogenicity was caused by a toxic effect. For their growth the *Rickettsia* preferred the protoplasm between the base of the cell and the nucleus. Even if only few *Rickettsiae* were present, the cell base showed vacuoles and other symptoms of degeneration, in the course of which shreds of cells or the entire mucous membrane became detached. This proved to be lethal for the lice. Invasion of the nuclei was never observed, not even with *rickettsiae* capable of propagating intracellularly in ticks or in tissue cultures. Strains of *R. conorii* from North, Kenyan, and Siberian tick-bite fever, furthermore strains of *R. akari* and *R. australis*, showed the lowest pathogenicity. Pathogenicity was highest with a strain of *R. rickettsii* from Mexico and a strain of *R. conorii* from North Africa. It has not been established whether the differences between the strains of *R. conorii* and *R. rickettsii* which were tested must be considered strain- or species-specific.

Unexpectedly, corresponding experiments on the transmission of *Rickettsia tsutsugamushi* (Hayashi) to lice failed [Weyer (88)]. The following five strains were tested: Kap (New Guinea), Gilliam (Burma), Korea, Kato and Hokkaido (Japan), and one of *R. saniyi* (Japan). Hence, this failure of transmission must be considered a species-specific property. The *rickettsiae* were not capable of invading the stomach cells. Evidently they were destroyed within a few hours after transmission or were voided with the feces.

The behavior of *Rickettsia* after intraocular transmission proved to be rather uniform. All strains tested, including the strains of *R. tsutsugamushi* as well as *R. quinella*, readily propagated in the hemolymph of lice and could be maintained through passages in lice for a considerable time by means of intraocular inoculation. Some species, e.g., *R. rickettsii*, grew better in the hemolymph than in the stomach cells. The fact that the *rickettsiae* multi-
plied mainly extracellularly is of biological importance. Only a few or them grow in hemocytes. In some instances (R. prowazekii, R. quintana, R. conori) invasion of the stomach cells from the hemolymph was occasionally found. Sometimes C. burnetii grew also in the cells of the hypodermis and the fat body.

R. prowazekii, R. typhi, and R. quintana are capable of multiplying in crab lice as well as in body and head lice [Weyer (84)]. The blood-sucking animal lice are probably also susceptible to R. typhi and R. prowazekii [Weyer (90)]. The occurrence of R. typhi in rat lice (Polyplax spinulosa), where the rickettsiae grow in the same manner as in body lice, has been demonstrated several times [Mooser, Castañeda & Zinsner (44)]. The susceptibility of monkey lice [Pediculus longiceps Piaget and P. albipes (Radov)] to R. prowazekii has been known for a long time [Blanc & Woodward (6)]. Weyer (83) inoculated pig lice (Haematopinus suis) rectally and intracoelomally with R. prowazekii. Invasion of the stomach cells followed exactly the same pattern as with body lice. The infection proved to be lethal for the lice. The rickettsiae multiplied readily in the hemolymph after intracoelomical inoculation, too. On the other hand, all experiments designed to render it possible for R. quintana to grow in the stomach cells or hemolymph failed. This agent seems to be a specific parasite of human lice.

The fact that the other rickettsiae, which are pathogenic for man, will grow in the stomach or in the hemolymph of lice, too, is of hardly any practical importance. Experimentally lice became infected by feeding on mice or guinea pigs. However, we do not know whether the rickettsiaemia of humans—suffering from rickettsialpox or tick-bite fever, for instance—will be sufficient to allow infection of lice, all the more since the amount of rickettsiae which are voided by infected lice with their feces is rather low as compared to an infection with R. prowazekii, R. typhi, or R. quintana. Demonstration of rickettsiae in lice under natural conditions, e.g., C. burnetii (Giroud & Jadin (26)) and R. rickettsii (Sylvia-Goyat & Elvisordo (68)), must be considered accidental. If rickettsiae are capable of reproduction in lice, this does not necessarily prove that transmission under natural conditions is possible. Although R. prowazekii and R. typhi will readily grow both in lice and fleas, rat fleas are the significant vectors of murine typhus, while body lice are responsible for the spreading of the classic epidemic typhus. Lice may play a role in the transmission of murine typhus only if this disease occurs epidemically.

The fact that the stomach and the hemolymph of lice are suitable culture media for various species of rickettsiae which differ considerably in their properties demonstrates the close bonds which exist between rickettsiae and arthropods, and indicates phylogenetic relationships between rickettsial species. If the behavior of rickettsiae in lice is used as a taxonomic base, R. quintana and R. tsutsugamushi will occupy exceptional positions. With R. quintana this results from its extracellular growth in the lumen of stomach and its requirement of human lice, while lack of ability to reproduce in the stomach justifies the exception of R. tsutsugamushi. The behavior of C. burnetii—abnormal development and the occasional invasion of organs—

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must be considered exceptional, too. The behavior of the remaining rickettsiae is generally in accordance with the classification of rickettsiae transmitted by ticks and insects. This classification indicates also the close relationships between R. prowazekii and R. typhi.

The biological relationships which exist between lice and rickettsiae are demonstrated by the ability of rickettsiae to survive for a considerable time and to multiply intensively in the stomach of lice. Evidently R. quintana is best adapted to lice, since the lice will not be harmed in any way by the infection. This is partly attributable to the extracellular reproduction of this agent. It is difficult to decide whether this relationship must be considered a primary or secondary adaptation. Furthermore, there are natural relationships between lice and R. prowazekii and R. typhi. Obviously the host-parasite association is not so old as that between rickettsiae and fleas, for rickettsial parasitism involves considerable injury of the lice [Mooser (41)]. Nevertheless, the adaptation is sufficient to ensure continuity of the species. The rickettsiae multiply intensively before the host becomes lethally injured. Sometimes there is even a balance between host and parasite, i.e., infected lice may harbor rickettsiae for life. Furthermore, large numbers of rickettsiae are voided with the feces where they may survive for several weeks, although rickettsiae are normally very sensitive [Chao (11); Starzyk (72); Weyer (90)]. R. quintana and R. prowazekii require lice and humans for their growth, the reservoir being formed by the latter; arthropod reservoirs do not exist. Late relapses of epidemic typhus (Brill-Zinsner disease) may lead to an infection of lice and thus to reappearance of the disease after very long intervals [Mooser (42); Murray & Snyder (49); Weyer & Horbourage (91)].

Ticks must be considered the original hosts of rickettsiae. These microbial agents will grow in ticks even if they are now normally transmitted by fleas and lice, which are probably secondary if not tertiary hosts. We do not know when in the course of evolution the group of rickettsiae (originally uniform, as it must be presumed) was split and divergent development began. The migrations of animals harboring rickettsiae after separation of the continents and the transition of these agents to other blood-sucking arthropods after a passage of warm-blooded hosts may have had a bearing on this phenomenon.

RELATIONSHIPS BETWEEN LICE AND SPIROCHETES

The biological relationships between lice and the agents of relapsing fever are clearly recognizable and must be considered perfect. Originally it had been assumed that lice were capable of transmitting only the agent of epidemic relapsing fever, Borrelia recurrentis (Lebert), but now it is a well-established fact that most species of Borrelia will survive and multiply in lice. Basic investigations on the behavior of Borrelia in lice were carried out years ago by Nicolle & Blanc (53) and Nicolle, Blaisot & Conseil (54). Further valuable observations were made by Chapheff (12), Chung & Feng (19), Sparrow (73, 74, 75), Toyoda (78), Wolman & Wolman (94), and others. Experiments on natural inoculation—by feeding lice on infected persons or animals—were supported by investigations on artificial inoculation [Baltazard
et al. (40); Mosler & Weyer (46); Sparrow (72); Weyer & Mosler (92). Some time after inoculation, the spirochetes will be abundant in the hemolymph of the lice, larvae as well as adults, where they readily multiply. They remain confined to the hemolymph and will not invade any organs, including sexual organs and salivary glands. Thus transmission by biting or any kind of transovarial transfer is impossible. The spirochetes can only leave their hosts together with the hemolymph—if this is lost after injury, for instance—and may then invade humans. A louse once infected will harbor spirochetes for life.

The percentage of lice which will become infected depends both on the species of *Borrelia* and on the amount of spirochetes ingested. The spirochetes reach the hemolymph, where their reproduction takes place, after active penetration of the stomach wall, but only few of them will succeed. With *B. recurrentis*, Chung & Feng (13) estimated that maximally 1 to 5 percent of the ingested parasites will reach the hemolymph. Some of the spirochetes are possibly voided with the feces while the louse is feeding, the majority, however, will die within a few hours in the arthropod's stomach. Only those spirochetes which succeed in reaching the hemolymph after passing through the stomach wall are of practical importance. Until recently the mode of this penetration was rather obscure. Most investigators believed that the spirochetes undergo a cyclic development in lice, during which they will disintegrate when passing the stomach wall, thus forming an invisible or hardly demonstrable stage, to reappear as "metacyclic spirochetes" in the hemolymph after 5 to 10 days. This assumption was maintained by Heisch (29, 40), Heisch & Garnham (31), Nicolle & Anderson (32), Nicolle & Lebailly (55), Sergent & Foley (66), and others. Their theory was mainly supported by the fact that no spirochetes were detectable in lice during the first days following the infection.

This hypothesis, which became questionable by the discovery that lice are infective during the "invisible stage," was based on inadequate methods of investigation [Mosler (43)]. When thoroughly examined, spirochetes will sporadically be found in the hemolymph of lice a few hours after the inoculation, i.e., those which succeeded in passing the stomach wall. These phenomena had already been described by Toyoda (78) and was later confirmed by Wolman & Wolman (94) and others. The spirochetes remain in lice without changing their shape, stability, mobility, and virulence. Their number is constantly growing by fission. Evidently their propagation rate increases after 5 to 7 days, the maximum being reached after approximately 10 to 12 days. The occurrence of the so-called metacyclic stage is actually the phase during which the spirochetes are so numerous in the hemolymph that they can be detected by less intensive methods of investigation. The appearance of this stage, as well as the number of positive lice, is dependent on the number of spirochetes which succeeded in penetrating the alimentary canal and on environmental conditions. These findings readily explain the contradictory experimental results. In cases of severe spirochetemia or inoculation of large doses, all lice will become positive.

The hemolymph of lice is a suitable medium not only for *B. recurrentis* but also for other species, which are naturally harbored by ticks. Baktazard et al. (40) were able to transfer the following species of *Borrelia* experimentally to lice: *B. microti* (Rafai), *B. turicata* (Brumpt), *B. meromiotes* (Blanc & Maurice), *B. parkeri* (Davis), *B. hermsii* (Davis), *B. hispanica* (de Buen), and *B. anserina* (Sakharral). Only *Borrelia persica* (Dchumowsky) proved to be an exception [Adler & Ashbel (1)]. Further successful transmission experiments were carried out with *Borrelia crocidurae* (Langer), *B. novyi* (Schellack), and *B. dipodilii* (Heisch). Particularly interesting are observations by Heisch & Garnham (31) and Heisch (29), who were able to demonstrate propagation of *Borrelia duttonii* (Novy and Knapp) in lice. Mosler & Weyer (46) maintained three strains of *B. duttonii* from Tanganyika continuously in body lice up to seven months without the spirochetes changing their properties. After rectal inoculation with diluted mouse blood 25 to 37.5 percent of the lice became positive, while up to 47.8 percent of the lice responded positively after natural inoculation by feeding on mice. A strain of *Borrelia turicata* (Brumpt) proved to be much less adapted to lice [Weyer & Mosler (92)]. On the other hand, a strain (a variety of *B. crocidurae*) which had been isolated from the small sub species of *Ornithodoros erraticus* (Lucas), yielded 100 percent positive lice after their feeding on mice.

Intraocoelic inoculation of spirochetes directly into the hemolymph of lice is followed by immediate reproduction. Thus, this method is particularly useful for maintaining those strains under laboratory conditions for which procuring of suitable animal hosts is difficult. It also represents a simple means of testing the propagation rate of various spirochetes in lice. Applying this technique, Gelgy, Mosler & Weyer (24) tested several East African strains of *B. duttonii* from *Ornithodoros monieuxi* (Murray) which had become avirulent for mice. These strains did not even multiply after direct inoculation into the hemolymph, though their viability and transferability to ticks remained unchanged for several weeks in the hemolymph.

According to the data available, which are rather incomplete, spirochetes—at least *B. recurrentis* and *B. duttonii*—are capable of reproduction not only in body lice but also in head lice. Corresponding observations on crab lice are still lacking, as are systematic or well-established studies on the behavior of spirochetes in animal lice, especially rat lice. Experiments have been carried out only on monkey lice, which also proved susceptible. On the other hand, *B. duttonii* did not multiply in pig lice, neither after rectal nor after intraocoelic inoculation [Mosler & Weyer (46)].

The biological relationships between lice and spirochetes are revealed by the spirochetes’ ability to survive in the stomach of the louse, to penetrate the stomach wall, to invade the hemolymph, and to multiply here without being affected themselves or harming their hosts. The better adaptation of *B. recurrentis* is apparently demonstrated by the fact that these spirochetes will reach the hemolymph more easily than other species. This ability to reach the hemolymph within a certain time seems to be a distinctive characteristic of the species of *Borrelia* which have so far been investigated. Only
one species, *B. farcinica*, is known to be lacking this ability. On the other hand, Helich. (29) was able to prove that body lice may be responsible for limited outbreaks of relapsing fever caused by *B. duttoni* in East Africa.

Penetration of the stomach wall is a decisive prerequisite to the growth of spirochetes and to their fate. We do not know as yet the factors governing this process. It is surprising how limited the chances are that spirochetes will reach a new warm-blooded host, as they are not voided with the feces, do not invade any organs, and are not transovarially transmitted. Their only way of transmission is via the hemolymph after injury of the lice, and it may be that some spirochetes are most abundant in the hemolymph of the legs, which break easily. This single mode of transmission is a significant distinction between the behavior of louse-borne and tick-borne spirochetes. Louse-borne spirochetes compensate for their small chance of finding a new host by their rapid propagation in lice, which will remain infected for life, and the large number of suitable hosts they find in cases of louseenese.

Concerning the origin of louse-borne relapsing fever, Nicolle and his collaborators offered the theory—which has been neither confirmed nor refuted—that this disease gradually developed out of tick-borne relapsing fever through adaptation of the agents to the cycle: man—louse—man (cf. Nicolle & Anderson (51)). This view is supported by the fact that tick spirochetes will readily multiply in body lice. It must be emphasized, however, that *B. recurrentis* definitely lost its ability to grow in ticks. Lice must be considered recent hosts as compared to ticks.

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