THE ROLE OF LICE IN TRANSMISSION OF SALMONELLA

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Following World War I, louse-borne diseases reached epidemic proportions in Europe. Particularly in Russia, in the years 1920 to 1922, when famine drove many people from the Volga region into Leningrad and other cities, relapsing fever and typhus became extremely prevalent. It was soon observed that many patients with relapsing fever developed an unusually severe disease, attended by high fatality, and leaving survivors with a multitude of complications including arthritis, osteomyelitis, gangrene, and abscesses in many organs. Early descriptions were published first in Russia and later in the German journals from which the present account is taken.

Iwaschenzoff and Kulescha and Titowa were among the first to identify the severe cases with a variety of paratyphoid infection which the former designated “N-Paratyphobazillose.” Among 167 cases of relapsing fever complicated by paratyphoid infection, he observed a fatality rate of 50%, whereas among 404 cases of uncomplicated relapsing fever the fatality was only 5.5%, and the similar rate for 100 cases of relapsing fever with various other complications was 10%. Kulescha and Titowa stressed the fact that the malignant form of paratyphoid infection occurred only in association with relapsing fever and specifically suggested that it originated from the bites of the same lice that carried the spirochetes. They stated that the latter hypothesis was under investigation, but they apparently lacked the materials and techniques to pursue this idea successfully.

Further bacteriologic studies showed that Iwaschenzoff’s paratyphus-N bacilli were of at least two types: N1, identical with the “Erzindjan bacillus” of Neukirch and now known as Salmonella paratyphi C; and N2, shown by Hicks to be a new type, related to Salmonella enteritidis, for which he and Bruce White proposed the name Salmonella, type Moscow.

Later, an association between S. enteritidis septicemia and relapsing fever was observed in China by several workers from the Peiping Union Medical Col:


lese,6-10 including Huang, Chang, and Lieu who suggested the "distant" possibility that the salmonellosis also was louse-borne.6 Liu, Zia, and Chung found S. enteritidis in cultures of lice removed from patients bacteremic with the same organism and undertook experimental work which indicated that S. enteritidis was capable of multiplication within human body lice and of producing fatal infection in guinea pigs when applied to scarified or even unbroken skin, thus demonstrating the possibility of louse-transmission.7 These investigations were continued by Liu11 who also studied the serologic properties of the strains of Salmonella involved.12 Further study by H. L. Chung of cases of Salmonella bacteremia occurring during an epidemic of relapsing fever led him to conclude that the human body louse was the vector of both infectious agents.9

More recently, Jadin13 and Kiyooka

The medical importance of louse-transmitted salmonellosis, if it occurs, is somewhat doubtful. On one hand, the relative infrequency with which circumstances indicating such a possibility have been noted and the diminishing incidence of pediculosis among human populations are reassuring in this regard. On the other hand, there has been renewed interest in louse-borne infections, especially in the Far East, where DDT-resistant lice occur commonly and where war and its aftermath have promoted widespread pediculosis. Furthermore, extant descriptions of certain historical epidemics fail to conform to the modern concept of any of the known plagues. It is possible that mixed infections, transmitted simultaneously, were responsible. The novel epidemiologic implications of the foregoing appeared to warrant a series of laboratory experiments designed to test the potentialities of the louse as a vector of Salmonella. In the studies reported here, human body lice were employed; one strain of S. enteritidis was examined intensively, and a few experiments were performed with other types of Salmonella.

MATERIALS AND METHODS
Source and maintenance of lice

The work of Culpepper in adapting human body lice (Pediculus humanus corporis) to feed on rabbits, and in continuously maintaining a colony of lice so adapted, has greatly facilitated experimentation with lice. All lice used in the present study were derived from a single shipment of eggs from the laboratories at Orlando. Lice were fed on rabbits twice daily, morning and afternoon, at least until the final moult from third instar into adulthood. Thereafter, those that were to be used in experiments were fed only once daily, and those from which eggs were to be collected for perpetuation of the colony were continued on the twice daily feeding schedule until a sufficient number of eggs had been deposited. Most locally obtained rabbits were found to be suitable for louse feedings.

Incubators for normal and infected lice were kept in separate rooms. Temperature of incubation was 32 C; the relative humidity in incubators varied from 25 to 35% but, since lice were kept in covered petri dishes, this latter observation would appear to be meaningless. The practice of controlling humidity in incubators by addition of water was soon abandoned.

In all experiments lice were used, without selection as to sex, as soon after the last moult as possible; thus the infecting meal was usually their first or second feeding as adults. These are hereafter designated "young adult lice."

 Cultures

Cultures dried in milk from the frozen state (lyophilized) were used throughout. For each experiment, a fresh tube from a single drying was tested for vacuum, opened aseptically, rehydrated and inoculated into 10 ml of heart infusion broth (Difco). After overnight incubation (16 to 20 hours), the culture was ready for use.

The following strains of Salmonella were employed:

- S. enteritidis S-795, from culture of tissue from a sick seal pup, C. ursinus.
- S. typhimurium 5609, originally isolated from spray-dried egg powder.
- S. pullorum A-LL, isolated locally from viscera of chicken.
- S. oranienburg Y-23, from stool of severely ill child involved in hospital outbreak of salmonellosis.
- S. paratyphi A, strains K2840, K2868, K2993, all recovered from prisoners of war in Korea.
- S. paratyphi A, M1103, isolated from patient in a New Orleans hospital. Supplied by Prof. M. F. Shaffer, Tulane University.
- S. typhi S10595, isolated locally from patient with typhoid fever.
- Shigella dysenteriae K-624 S, laboratory stock strain, was also used.

Membrane feeding technique

The chick membrane feeding technique of Fuller, Murray and Snyder was employed with

* Bureau of Entomology and Plant Quarantine, Agricultural Research Administration, U. S. Department of Agriculture, Orlando, Florida.

Skin was always freshly obtained from the youngest available white Leghorn chicks that would supply membranes of the required size (usually 1 to 3 days for small cylinders, 2 weeks for large; see below). Down was clipped mechanically. Disinfection was not attempted. Membranes were tied with thread over open ends of glass cylinders of two sizes. Up to 150 lice were fed in cylinders with inside diameter of 18 mm, larger numbers in cylinders of 41 mm diameter. Feeding periods of 30 minutes were employed unless otherwise stated.

Blood meals were prepared in beakers just enough larger than the cylinders to accommodate the latter readily but still hold them upright. Dilutions of culture were prepared in M/10 phosphate-buffered physiological saline, pH 7.2 (buffered saline), and the last decimal dilution was made in defibrinated rabbit blood. Since the number of viable Salmonella in the overnight broth culture, as determined by plate counts, was always close to 1 x 10^9 per ml, knowledge of the average amount of blood ingested per louse made it possible to adjust the average dose, in terms of numbers of bacteria, within reasonable limits.

The lack of a sterile membrane was no handicap in work with Salmonella, since adventitious bacteria rarely seemed to multiply in the lice and the availability of highly selective media with differential properties made it possible to perform accurate plate counts and standard isolation procedures in the presence of airborne and skin contaminants.

RESULTS

Animal experiments.—Preliminary experiments were performed in which lice were first exposed to S. enteritidis by allowing them to feed on infected rabbits and then, at intervals, were refed on normal rabbits to test their ability to transmit the organism. The average number of viable Salmonella per louse meal was determined by removing a sample of lice (10 to 25) to a mortar immediately after feeding, triturating with 0.1 ml buffered saline per louse, and spreading replicate SS agar plates with 0.1 ml aliquots of the resulting suspension and dilutions thereof. The concentration of Salmonella in lice and louse feces at the time of refeding was similarly determined. Although rabbits vary considerably in their susceptibility to salmonellosis, large doses (about 10^7 bacteria) of S. enteritidis S-795, given by both the intravenous and subcutaneous routes at the same time, consistently rendered mature rabbits infectious to lice for a period beginning within 24 hours after inoculation and lasting until death of the animal 1 to 3 days later. The pathogenicity for lice of strain S-795 (S. enteritidis) was so much greater than expected that early attempts at transmission were largely invalidated. Most lice taking an infectious meal in the afternoon were dead by the next morning, and the survivors were so debilitated that few were able to feed. Observation of infected lice with the aid of a dissecting microscope revealed increased activity of the intestinal tract (as compared with normal lice), frequently leading to rapid purgation. Samples of lice were macerated and triturated at intervals after the ingestion of an infectious meal containing, on the average, less than 100 viable Salmonella; within 5 to 7 hours the bacteria had attained a level ranging from 10^4 to more than 10^6 viables* per louse. After such intervals the remaining infected lice still would bite and feed avidly.

It was also found that the feces of infected lice contained up to 10^6 viable Salmonella per mg. Since lice usually defecate while feeding, this was a factor to be considered in transmission experiments. In several trials, after infected

* Here and elsewhere the term “viable” is used as a substantive to signify the unit in which results of plate counts are expressed. A short expression for the cumbersome “viable bacteria as determined by plate counts” is needed, especially in tables.
Salmonella in Lice

Table 1.—Attempted louse-transmission of S. enteritidis from infected to normal animals.

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of lice</th>
<th>Bacteriemia in rabbit at time of feeding (viables per ml blood)</th>
<th>Interval to refeeding (hours)</th>
<th>Viables per louse</th>
<th>Species of normal animal</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>88</td>
<td>$8.7 \times 10^8$</td>
<td>5</td>
<td>$10^4$</td>
<td>Rabbit</td>
<td>Negative</td>
</tr>
<tr>
<td>B</td>
<td>350</td>
<td>$4.4 \times 10^9$</td>
<td>7</td>
<td>$5.2 \times 10^9$</td>
<td>Rabbit</td>
<td>Negative</td>
</tr>
<tr>
<td>C</td>
<td>250</td>
<td>$3.4 \times 10^9$</td>
<td>16</td>
<td>$10^7$</td>
<td>Rabbit</td>
<td>Negative</td>
</tr>
<tr>
<td>D</td>
<td>100</td>
<td>$1.8 \times 10^9$</td>
<td>24</td>
<td>$10^4$</td>
<td>Rabbit</td>
<td>Negative</td>
</tr>
<tr>
<td>E</td>
<td>200</td>
<td>$5.5 \times 10^9$</td>
<td>6</td>
<td>$2.0 \times 10^9$</td>
<td>Mouse</td>
<td>Doubtful</td>
</tr>
<tr>
<td>F</td>
<td>200</td>
<td>$5.5 \times 10^9$</td>
<td>24</td>
<td>$5.2 \times 10^9$</td>
<td>Mouse</td>
<td>Doubtful</td>
</tr>
</tbody>
</table>

* The multiplier of $10^9$ is about the average number of bacteria ingested per louse.
† Approximate value.

Lice had been removed, the louse feces were washed off and the rabbit's belly disinfected externally in order to favor transmission by bite alone, if this were possible. In others, the feces were rubbed into the mildly scarified skin before mechanical removal of excess.

Representative data obtained by animal experiments are given in table 1. In no case did rabbits become infected through the feeding of infected lice, although fatal infection could easily be produced by injection of suspensions of the same lice. Since relatively large doses of the test culture were required to produce recognizable infections in rabbits, another animal might be more suitable if the lice could be induced to bite. It was found that they would bite suckling mice and feed to a limited extent. Also, mice were known to be very susceptible to infection with the test culture by any of several routes of inoculation.

Lice were, therefore, infected on bacteremic rabbits; then, after 6 and 24 hours, they were induced to feed on suckling mice (Groups E and F, table 1). One of the baby mice died on the sixth day after exposure, and S. enteritidis was isolated from heart's blood, intestinal contents, liver and spleen. However, within the succeeding 4 days all remaining mice, including nonexposed litter mates and their dams in each jar, died of salmonellosis. In the light of extensive earlier experience with the test culture in mice, these observations could not be interpreted as evidence for direct transmission of Salmonella from infected lice to the mice which were bitten. All dams died 7 to 11 days following exposure of their progeny. This is the usual survival time of adult mice given a moderately large peroral dose of the culture in question. All baby mice died within approximately the same interval, which is a much longer survival time than would be expected after parenteral inoculation with even very small doses of the same culture.* The mother mice were, in fact, perceptibly ill before clinical signs appeared in their young. It may be reasonably concluded that the mothers ingested infective doses of Salmonella by licking louse feces from the young as they were returned following exposure to lice, then transmitted the organism to the suckling mice either through the milk or by fecal contamination.

Membrane feeding experiments.—Since these relatively crude animal experiments left much to be desired, very little information regarding the possibility of human to human transmission of Salmonella by lice was gained. It appeared that an artificial system might yield more precise data capable of

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Milner, K. C. Unpublished data.
broader application. Such a system was available in the chick membrane feeding technique of Fuller, Murray and Snyder. With only slight modification and extension, this technique proved ideal for work with bacteria of the enteric group since the existence of highly selective media eliminated one of the chief drawbacks of the method.

**Determination of minimum infectious dose.** — With this technique an accurate titration of the infectious dose of *Salmonella* for lice became possible. In order to obtain data such as those presented in table 2, several preliminary experiments were required to establish the useful range of dilutions, and to acquire speed and facility in the technical manipulations. When all was in readiness, three fresh chick membranes were prepared and serial decimal dilutions in buffered saline were made from an overnight broth culture of the test organism. The membranes were placed in contact with blood meals consisting of 2.0 ml of appropriate dilutions of culture thoroughly mixed with 18.0 ml of defibrinated rabbit blood, and mounted in the water bath. A circular pad containing about 150 lice was then dropped onto each membrane and allowed to remain for 30 minutes. The succeeding operations were performed rapidly and as nearly simultaneously as possible. Fifty lice that had obviously fed to repletion in each group were counted into mortars and triturated with 0.1 ml buffered saline per louse; the remaining fed lice were returned to the incubator. Such dilutions of the louse suspension, the blood meals, and the original broth culture as appeared necessary were then prepared and 0.1 ml aliquots were spread on 3 to 6 replicate plates of brilliant green agar for colony counts.

The figures in the second and third columns, table 2, were calculated from the plate counts on the assumption that lice had ingested an average of 1 ml of blood. The figures in column 4 are the averages of 3 replicate plates, each spread directly from 0.1 ml aliquots of the emulsions of fed lice, and they represent the average numbers of viable *Salmonella* per louse immediately after the 30-minute feeding period. The essential agreement between the 3 methods, which is even closer if columns 2 and 3 are corrected for the experimentally determined size of blood meal (see below), indicates that any one is a reasonably accurate method for determining the size of dose. It is also evident from this and several similar experiments that the majority of lice ingesting even one viable cell of strain S-795 might be expected to develop fatal infection with *Salmonella*.

**Multiplication of *S. enteritidis* in lice.** — Results of one attempt to follow the multiplication of strain S-795 in lice after ingestion of an infectious dose are recorded in table 3. After allowing lice to feed through a chick membrane on a

| Table 2.—Titration of infectious dose for lice of *S. enteritidis*, S-795 |
|------------------------|-------------|-----------------|-----------------|
| Group                  | Dil of culture | Blood meal | Sample of 59 fed lice | Percent of re- | No. of viables per dead louse |
| A(114)                 | 6.10         | 58.00       | 48                | 90             | 8.3×10^6 |
| B(154)                 | 6.10         | 55.63       | 48                | 90             | 1.5×10^7 |
| C(160)                 | 6.01         | 0.51        | 40                | 40             | 4.7×10^6 |

*Numbers in parentheses are numbers of lice in each group which were observed to have fed to repletion. Those which fed poorly were removed from the pads and discarded.*

| Table 3.—Rate of multiplication of *S. enteritidis* in the louse. * |
|------------------------|-----------------|-----------------|
| Hours after feeding | No. of lice sampled | No. of viables per louse |
| 0                      | 25              | 1.1×10^6 |
| 7                      | 20              | 6.6×10^6 |
| 4                      | 20              | 7.9×10^6 |
| 6                      | 20              | 1.4×10^6 |

*Lice infected by allowing them to feed through membrane on blood containing about 10^6 viables per ml.*
preparation designed to provide approximately 100 viables per louse meal, samples of lice were removed for titration immediately and at 2, 4, and 6 hours thereafter. Throughout this period, the numbers of *Salmonella* per louse increased almost logarithmically, with very slight lag. Although repeated tests showed moderate variation in the rate of multiplication, generally groups of lice receiving larger infecting doses had correspondingly higher concentrations at the end of 6 hours. Thus, if the appropriate groups from tables 1, 3, and 4 are arranged in order of increasing dose, it will be seen that average meals of 55, 110, 620, and 6200 viables resulted, at the end of 6 hours, in viable counts of $2.6 \times 10^7$, $1.8 \times 10^9$, $5.6 \times 10^6$, and $1.1 \times 10^7$, respectively. However, the data in table 2 (column 6) suggest that lice receiving minimal infecting doses may eventually develop higher concentrations than lice ingesting greater numbers of bacteria.

**Average amount of blood ingested.**—The size of the average blood meal of lice as we used them was determined by weighing. One hundred "young adult lice," not separated as to sex (see *Materials and Methods*), were placed in a weighing bottle and weighed to the nearest 0.1 mg 18 hours after their last previous meal. The average weight per louse was found to be 1.248 mg. These lice were then placed on a chick membrane in contact with defibrinated rabbit blood under the usual conditions and allowed to feed for 40 minutes. The lice which had obviously engorged were then reweighed and found to average 2.134 mg each. The difference of 0.886 mg was the average weight of blood ingested. In terms of volume, this would be about 0.0084 ml. Individual variations were not determined, although such measurements have since been reported by Fuller.23 It appeared that assigning the convenient value of 1 μl to the average louse meal would not seriously distort most of the results.

**The possibility of transmission by bite.**—Although lice are not known with certainty to transmit any pathogen directly through the bite, it seemed important to test this possibility in the case of *salmonellosis*. Experiments with rabbits and mice had been inconclusive in this regard, but use of the chick membrane feeding technique "in reverse," so to speak, offered one possibility of quantitative measurement. Infected lice feeding through a membrane on sterile blood should leave some bacteria in the blood on the sterile side of the membrane if transmission by bite were indeed possible; and, of course, the numbers of such transferred bacteria could be readily determined by standard methods. Use of a similar procedure involving mosquitoes, bat-wing membranes, and viral agents has since been reported by Ross.24

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24. Ross, R. W. 1956, A laboratory technique for studying the insect transmission of animal
Table 4 summarizes the results of 1 of 7 similar experiments which were performed. All infected lice of groups A and B appeared to "bite" through the membranes and ingest at least some blood when given the opportunity to refeed. After a 45-minute feeding period, 20 infected lice from each group were sacrificed and titrated. Six portions, totaling 12.5 ml, from each 20 ml preparation of blood were cultured and found uniformly free of Salmonella. Titration of lice sampled at the time of refeeding indicated that all lice were heavily infected at that time, and all were dead when examined 24 hours after the infectious meal. Their bodies then yielded 3-6 x 10⁷ viable Salmonella per louse.

In some experiments, one or more preparations of normal blood yielded Salmonella after infected lice had fed on it through a chick membrane. In each such case, there were imperfections in the membrane (apparently due to pin feathers) which permitted blood to seep through to the side the lice were on, and which might have permitted bacteria from feces to pass the other way in limited numbers. (The slight pressure caused by the weight of the cylinder and stretched membrane floating on the blood would tend to minimize chance transfer of materials in the direction of the blood, even in the presence of imperfections, but this gradient could be overcome, on occasion, by the motility of the Salmonella.) In no instance did as many as 50 viable Salmonella reach the normal blood, despite the feeding of up to 200 lice in various stages of infection. Experimental inoculations showed that this would not constitute an infectious dose for a rabbit or guinea pig even though such a number had been actively transferred by the lice. The bulk of our evidence, therefore, indicated that lice were unable to transmit Salmonella directly by bite.

Infectivity of other cultures for lice.—The infectivity for lice of certain cultures other than S. enteritidis S-795 also was tested. Table 5 gives the results of chick membrane feeding experiments with S. typhimurium, S. oranienburg, S. pullorum, S. typhi, and several cultures of S. paratyphi A. All cultures were infective for lice but not equally lethal. Lice infected with S. typhi, for example, were active and able to feed well 24 hours after an infectious meal despite an average concentration in their bodies at that time of over 10⁶ viable typhoid bacilli per louse. One culture of Sh. dysenteriae (K624) was tested and found to be weakly pathogenic although able to survive and multiply in the lice. The majority of lice infected with it were alive and active 48 hours after the infectious meal despite starvation for that interval.

Survival of Salmonella in dead lice and in louse feces.—From the foregoing it is
evident that ingestion of viable *Salmonella* of any of several types leads to fatal infection in lice and that, although the time until death varies with the culture, the lice, at death, each contain about $10^7$ viable *Salmonella* in their bodies. It was also determined that freshly passed feces of lice infected with *S. enteritidis* contained on the order of $10^6$ viable *Salmonella* per mg. The survival of these microorganisms in dead lice and louse excreta under various conditions of storage would have a bearing on the possible role of lice in spread of salmonellosis. These matters were not investigated systematically, but the scattered observations summarized in table 6 show that at least *S. enteritidis* and *S. typhi* may be preserved in viable condition in the bodies and excreta of lice for an extended period. Storage in all cases was in ordinary petri dishes at normally fluctuating room temperature. The relative humidity was usually under 30%. The feces were collected from dishes which had housed lice before and after an infective meal; therefore, a considerable admixture of normal material was present in the samples tested.

Most of the same lots were retested after an interval of 3 to 4 years, at which time only 2 lots of lice (K 1 and K 2, table 6) and one of louse feces (L 2, not tested previously) yielded viable *Salmonella*. Material from lot P 1, which preserved the pathogen remarkably well for 13 months, failed to yield *S. typhi* after 3 years of storage under the same conditions.

**Effect of passage through lice on mouse virulence of *S. enteritidis***. Passage of *Salmonella* through lice might conceivably alter the virulence of the bacteria and, as a consequence, the vector potential of lice might be greater or less than would appear from the foregoing experiments. Such a hypothesis was tested in one admittedly inconclusive experiment. Lice were infected through a membrane with average doses of $7 \times 10^7$ viable *S. enteritidis S-795*. The following day, 50 dead lice (averaging $2 \times 10^7$ viables per louse) were triturated with saline in a mortar, and the resulting suspension was diluted serially. Various dilutions also were made of the usual type of overnight broth culture, and mice were injected intraperitoneally with 0.1 ml aliquots of the preparations. The effects of doses, from infected lice and from culture, which matched most closely are compared in table 7. We do not believe that the figures show any clear-cut evidence of altered virulence of the *Salmonella* following one passage through lice. Additional passages were not performed because serial passages through lice would not be likely to occur naturally.

**The lethality for mice of combined infection with *Salmonella* and *Borrelia***. Since cases in human beings of relapsing

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**Table 6.** Survival of *S. enteritidis* and *S. typhi* in dead lice and in louse feces.

<table>
<thead>
<tr>
<th>Lot No.</th>
<th>Organism</th>
<th>Interval to test</th>
<th>No. of viable <em>Salmonella</em></th>
<th>Lice</th>
<th>Per louse</th>
<th>Per mg louse feces</th>
</tr>
</thead>
<tbody>
<tr>
<td>I 1</td>
<td><em>S. enteritidis</em></td>
<td>1 day</td>
<td>$5.7 \times 10^6$</td>
<td>3.7</td>
<td>10^6</td>
<td>-</td>
</tr>
<tr>
<td>I 2</td>
<td><em>S. enteritidis</em></td>
<td>8 days</td>
<td>9.0 \times 10^6</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>I 3</td>
<td><em>S. enteritidis</em></td>
<td>23 days</td>
<td>5.1 \times 10^6</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>I 4</td>
<td><em>S. enteritidis</em></td>
<td>10 months</td>
<td>5.7 \times 10^6</td>
<td>3.7</td>
<td>10^6</td>
<td>-</td>
</tr>
<tr>
<td>K 1</td>
<td><em>S. enteritidis</em></td>
<td>16 months</td>
<td>5.0 \times 10^6</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>K 2</td>
<td><em>S. enteritidis</em></td>
<td>16 months</td>
<td>6.3 \times 10^6</td>
<td>1.8 \times 10^7</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>L 1</td>
<td><em>S. enteritidis</em></td>
<td>17 months</td>
<td>5.8</td>
<td>3.8 \times 10^6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>L 2</td>
<td><em>S. enteritidis</em></td>
<td>19 months</td>
<td>5.5</td>
<td>3.8</td>
<td>10^6</td>
<td>-</td>
</tr>
<tr>
<td>P 1</td>
<td><em>S. enteritidis</em></td>
<td>20 months</td>
<td>5.0</td>
<td>3.8</td>
<td>10^6</td>
<td>-</td>
</tr>
<tr>
<td>P 2</td>
<td><em>S. enteritidis</em></td>
<td>13 months</td>
<td>5.8</td>
<td>1.2 \times 10^6</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* Not done. In other experiments, ca. 10^6 viable *S. enteritidis* per mg freshly passed feces from infected lice.

**Table 7.** Effect of passage through lice on mouse virulence of *S. enteritidis S-795*.

<table>
<thead>
<tr>
<th>No. of mice</th>
<th>I.P. dose (viables)</th>
<th>Source</th>
<th>Mean survival time in days (limited to 21 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>1,700</td>
<td>Lice</td>
<td>6.5</td>
</tr>
<tr>
<td>20</td>
<td>1,300</td>
<td>Culture</td>
<td>6.5</td>
</tr>
<tr>
<td>20</td>
<td>1,700</td>
<td>Lice</td>
<td>10.5</td>
</tr>
<tr>
<td>20</td>
<td>1,300</td>
<td>Culture</td>
<td>10.5</td>
</tr>
<tr>
<td>20</td>
<td>17</td>
<td>Lice</td>
<td>13.5</td>
</tr>
<tr>
<td>20</td>
<td>13</td>
<td>Culture</td>
<td>11.5</td>
</tr>
</tbody>
</table>
fever complicated by salmonellosis had been reported to be exceptionally severe, a laboratory comparison of these infections singly and in combinations was attempted. In controlled experiments in which mice were first inoculated, parenterally, with relapsing fever spirochetes (derived from the small form of Ornithodoros erraticus, collected in Egypt) and at intervals later inoculated perorally with infectious doses of strain S-795, fatality rates and duration of survival depended solely on the inoculum of Salmonella. Relapsing fever was not, of itself, lethal to mice; and mice exposed to Salmonella alone died as rapidly as mice inoculated with both agents.

By contrast, when both agents were given simultaneously by parenteral routes, there appeared to be an effect of enhancement (table 8). The mean survival time of 96 mice so inoculated was significantly lower than that of 48 mice injected with S. enteritidis only (p < .001). The one death which occurred among 48 mice inoculated with Borrelia of relapsing fever alone was probably nonspecific.

**DISCUSSION**

For lice to play any important role in transmission of microorganisms it is necessary that they first feed upon an individual whose blood contains a sufficient concentration of the agent to provide a louse infectious dose in the small volume of a louse meal. In the case of Salmonella, this minimum effective concentration would be on the order of 1000 bacteria per ml. Although the majority of patients with salmonellosis do not have organisms in the blood, those with the enteric fever or septicemic types of the disease frequently develop the necessary degree of bacteremia. S. enteritidis is regarded as, typically, an agent of food infection (gastroenteritis), but both sporadic cases and epidemics of a generalized form of infection with this organism are known to occur.

The present studies show that, once infected, the louse becomes a potent source of infection for man. That lice would be able to transmit infection directly through the bite was not expected, since none of the established louse-borne diseases appear to be spread in this fashion; but the development of high concentrations of Salmonella in the bodies and excreta of lice makes it possible for them to transmit these bac-

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bacteria by the same means by which they transmit the agents of typhus and relapsing fevers. Furthermore, the presence of infected lice would add materially to the hazards of ingesting infectious doses of Salmonella. Food might be easily contaminated by dead lice or louse excreta; and the probable consequences of killing infected lice by crushing them between the teeth are obvious.

Since the lice under study usually partook of a sterile meal, it is perhaps not surprising that microorganisms were not numerous in stained smears from normal lice. However, some were present; and it is scarcely conceivable that in the process of penetrating a nonsterile membrane lice would be able to avoid ingesting bacteria from the normal skin flora of the host animal. Such adventitious bacteria ordinarily do not multiply greatly. The enormous multiplication of Salmonella with attendant purgation and lethal effect indicates a true pathogenicity of these bacteria for lice rather than an accidentally favorable culture medium in the blood meal only. This is supported by the experimental finding that counts of Salmonella in lice that appeared to be completely purged did not differ significantly from those in lice, of the same group, which had died with a considerable residuum of blood in the gut.

This investigation was interrupted by accidental loss of the louse colony and other activities have prevented its resumption. Among several objectives not realized were studies of the range of infectivity and pathogenicity of bacteria for lice and a histologic study of the development of S. enteritidis within the louse. In a few preliminary microscopic observations, stained smears from crushed normal lice showed few or no bacilli, whereas similar preparations from infected lice revealed many bacteria morphologically compatible with Salmonella. Also, when efforts were made to dissect out the apparently intact gut of lice dead of Salmonella infection and stain it separately from the hemolymph, all smears of gut showed heavy concentrations of bacilli, as did some of the smears of hemolymph. Some of the latter, however, showed few or rare bacteria. Taken in conjunction with the finding of many viable Salmonella in homogenates of purged, infected lice, this may indicate that, as in typhus, the louse tissue most heavily invaded is the epithelium of the gut, whence the pathogens are shed into the lumen to be passed with the feces, or may also escape into the body cavity to produce generalized invasion. The above findings in no way depend upon the rather frequently observed rupture of a louse gut during feeding with liberation of the blood meal into the body cavity and early death of the insect; such lice were always removed from the experiment.

The type of salmonellosis to which this discussion pertains is not common nor likely to occur in ordinary civilian life. Aside from the observations of the Peiping group (made on beggars and dope addicts, under conditions of exceptional crowding and untidiness), all applicable records concern military personnel or civilian groups disorganized by war, but those records describe a salmonellosis of frightening severity with frequent complications among survivors. The observations of many Russian scientists during the “difficult times” following World War I and the revolution, of Savino and Menendez in Paraguay at a time of military action, of Hayes and Freeman in India, and of Neukirch in Turkey all refer to the type of disease under consideration here. Whether or not louse trans-

30. Neukirch, P. 1918, Über menschliche Erkran-
kungen durch Bazillen der Gläser-Volldag-
Infektionskrankh. 85: 103-145.
mission occurred cannot be proved, but the people involved almost certainly harbored lice (as attested by observers as well as by the concomitant prevalence of relapsing fever or typhus), and the present laboratory studies show that this possibility cannot be ignored. The extreme malignance of such infections may have been due to the operation of additional stresses such as malnutrition, exposure, or complication with other infections; to the presence of microbes of exceptional virulence; or to inoculation by a parenteral route rather than the usual fecal-oral chain of infection.

SUMMARY

The possibility that salmonellosis might be, under some conditions, a louse-borne disease has been investigated with the aid of human body lice maintained on rabbits and fed experimentally through membranes of fresh chick skin.

Lice ingesting even one viable unit (as determined by plate counts) of Salmonella enteritidis (strain S-795) became infected and eventually developed $0.5-5.0 \times 10^7$ bacteria in their bodies; about 90% were dead in 24 hours and all died within 48 hours. The bacteria developed logarithmically in the lice between 2 and 6 hours after introduction of an infectious dose, and reached maximum titer in 6 to 8 hours. Viable Salmonella survived in lice and louse feces in considerable numbers for more than one year and in exceptional cases for as long as 4 years. Cultures of several other types of Salmonella, including Salmonella typhi were found to be similarly infectious for lice, although not all were as rapidly lethal.

One passage of S. enteritidis through lice did not materially alter the virulence of the culture for mice. The virulence for mice of mixed infections with S. enteritidis and Borrelia of relapsing fever was the same as for the Salmonella alone when the latter was introduced perorally, but when both agents were introduced by parenteral routes at the same time, a significant enhancement of virulence was noted.

Lice became infected when permitted to feed on bacteremic rabbits, but failed to transmit Salmonella to normal rabbits by feeding. However, when the same lice were macerated and injected parenterally into other rabbits, fatal infection ensued. Although suckling mice that had been bitten by infected lice became infected, these mice probably acquired the disease indirectly through the agency of louse feces. Membrane feeding experiments also indicated that lice were unable to transmit Salmonella directly by bite. Therefore, if lice become infected by feeding on an individual with a bacteremia in excess of $10^3$ bacteria per ml, they have the same potential for transmitting Salmonella as, under analogous circumstances, they would have for transmitting the agent of typhus.