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A MICROSCOPIC STUDY OF *PASTEURELLA TULARENSIS* IN THE HUMAN BODY LOUSE*

By ROGER D. PRICE, Ph.D.

Department of Entomology and Economic Zoology, University of Minnesota, St Paul

(With Plate XVIII and 1 Figure in the Text)

INTRODUCTION

This paper presents the results of the third phase of an investigation into the relationship between the tularemia organism, *Pasteurella tularensis*, and the human body louse, *Pediculus humanus humanus* L. These studies dealt with a thorough histological study of lice in various stages of infection to determine the fate of *P. tularensis* in the louse.

In a qualitative study on the ability of the tularemia organisms to survive in lice and louse faeces, it was shown that both substrates harbour the organisms for varying periods ranging up to 53 days (Price, 1954). It was subsequently demonstrated that the human body louse is susceptible to infection with *P. tularensis*, the percentage retaining infection over a period of time depending upon the number of organisms accessible to the louse in its blood meal (Price, 1956). By using quantitative methods, it was noted that an increasing percentage of the lice lost its infection with passage of time, whereas in some of the lice the bacteria multiplied extensively.

A histological examination of this nature is important, both to supplement the picture of louse infection obtained in the previous works and to afford a means of comparison with lice infected with the various rickettsiae pathogenic to man. Fundamental to the definition of rickettsiae is the intimate association with arthropod tissues, and *P. tularensis* is the only bacterial pathogen of humans which shows a predilection for intracellular development within the arthropod host. Since Weyer (1954) has made a series of comprehensive histological studies of *Pediculus humanus humanus* infected with the rickettsiae pathogenic to humans, it is of interest to determine in what respects an infection of body lice with *Pasteurella tularensis* resembles these findings.

Only one other study of tularemia in the tissues of arthropods is known to the writer. Francis (1927) found ample cellular infection in the epithelium of the digestive tract and occasionally in the Malpighian tubules of *Dermacentor andersoni* Stiles and *Cimex lectularius* L.

MATERIALS AND METHODS

The strains of body lice, *Pasteurella tularensis*, and white mice used throughout the study were the same as used previously by this worker (Price, 1954). The agar used for the cultivation of this organism was glucose-cysteine blood agar (GCBA)

* Contribution no. 918 from the Department of Entomology, University of Kansas, Lawrence.

prepared essentially after the method of Downs, Coriell, Chapman & Klauber (1947); in order to reduce the growth of bacterial contaminants, 6250 units of penicillin and 25 mg. of sodium sulphadiazine were added to 500 ml. of medium.

Lice were infected by the method of Snyder & Wheeler (1945), utilizing the marginal ear vein of a rabbit for an intravenous inoculation of a massive dose of the infectious organisms. All lice used were starved for 12–24 hr. before infection. They were applied to the rabbit immediately after the injection of the infectious material and allowed to feed for 20–30 min. After this time the lice were removed and all lice which obviously had not fed were discarded. The infected lice were stored in desiccating jars at 29° C. and a relative humidity of 60 %, using the sulphuric acid method of Solomon (1951) for humidity control.

A standard procedure was adopted for the quantitative determination of the number of organisms in each louse. The individual louse was placed in a sterile mortar with 1·5 ml. sterile saline and thoroughly macerated. This original suspension was considered as a 10° suspension, and 1·0 ml. of it was transferred to a 99 ml. saline blank, with 0·2 ml. being used to make duplicate 10° plates, 0·1 ml. per plate. Duplicate plates similarly were made at 10^{-2} and a further 100-fold dilution enabled duplicate plates to be made at the 10^{-4} dilution. When pooled lice were plated, a similar procedure was adopted. For all plate counts, the GCBA plates were incubated at 37° C. for a minimum of 3 days before being counted. The accuracy of this plate-count method was tested statistically and found to be adequate (Price, 1956).

The infected lice were fed once daily on normal rabbits, this interval being kept as near 24 hr. as was possible. No rabbit was used for more than the 48 hr. period following the first feeding of infected lice. In this way one rabbit would provide at most three consecutive meals. To test for a possible early circulation of *P. tularensis* each rabbit was bled from the marginal ear vein immediately before the second and third feedings of the lice. Two white mice were inoculated with 0·5 ml. each of this blood. The mice were observed daily until the fifteenth day at which time, if still living, they were sacrificed and autopsied. The lymph nodes, spleen, and liver of every mouse were examined grossly for pathological changes indicative of tularemia and plate smears of the spleen and heart blood were made. The rabbits were similarly observed and autopsied after infection-induced or sacrifice-induced death. Gram-stain preparations were made of any apparently positive plates from these animals.

All lice used for the preparation of serial sections were fixed in Gilson's fixative after they were killed by a 5 sec. immersion in boiling water. The heads and legs were removed after killing and before fixation. The method of dehydration and embedding was based mainly on the *n*-butyl alcohol technique of Stiles (1934). At the suggestion of Dillon (1954), phenol was added to the higher alcohol solutions to give a concentration of 4 %.

The lice were cross-sectioned at 4 or 5μ thickness. The usual method of affixing the tissues to the slides was followed with the exception of the substitution of a rabbit blood serum adhesive (Priman, 1954) for the egg albumin formula.

Sections were stained in Giemsa stain following Lee (1950), whose procedure was altered by reducing to 20 drops the amount of Giemsa stock and increasing to 4·0 ml. the volume of pure methyl alcohol for each 50 ml. distilled water. The slides were left in the stain for only 4–6 hr., followed by a 20–25 min. differentiation in 95 % ethyl alcohol.

MICROSCOPIC STUDY OF INFECTED LICE

Approximately 5000 lice of mixed ages were infected to obtain material for the preparation of serial sections. To ascertain the course of the infection within these lice, counts of pools of five lice were performed. Ten pools tested on the initial day were 100 % infected with a maximum of 16×10^6 and a minimum of 6×10^6 organisms per pool, the average being 10×10^6. Ten pools tested on the seventh day after infection were still 100 % infected, the maximum being 80×10^6, the minimum 5×10^6, and the average 27×10^6 organisms per pool. Only five pools were tested on the fourteenth day, 80 % of these being infected with a maximum of 20×10^6 organisms per pool. These results indicated some bacterial multiplication within the lice of this infected colony.

On the sixth and thirteenth days, 27 and 25 female lice, respectively, were placed individually into faecal collectors after their meal for that day. The 24 hr. accumulation of faeces for each louse was ground individually in 0·6 ml. sterile saline and the undiluted suspension plated in duplicate on GCBA plates, 0·1 ml. per plate. Faeces and lice were numbered correspondingly, and the latter were fixed and dehydrated individually. The lice were then grouped into three groups depending on whether the plates made from their faeces exhibited: (1) a growth too heavy to count easily (23 lice); (2) 1–300 colonies per plate (21 lice); (3) no *P. tularensis* colonies (eight lice). Ten lice, randomly selected from each of the first two groups, and the eight lice of group 3 were sectioned.

All of the lice examined from the group with heavily infected faeces showed excellent development of tularemia-like organisms in the epithelium of the midgut. Of the total of 3203 sections inspected, 376, or 11·7 %, showed intracellular infection with these organisms. Of the 10 lice from the second group, seven showed infected gut cells, and of these seven, only two had as extensive infection as the preceding group. Only 116, or 3·9 %, of the 2981 sections of these lice revealed infected cells. Significantly enough, all 2134 sections of the eight lice examined from the uninfected-faeces group showed no cells with tularemia-like organisms. Since the above sections were cross-sections, it should be emphasized that in the heavily infected lice there was more infection along the lateral axis as well as the longitudinal axis.

Lice for the main histological study were selected from the colony of infected lice maintained for a period of 35 days (Price, 1956). Their manner of selection was as follows: 19 lice on the first day, 21 on the fourth day, 25 on the tenth day, 27 on the seventeenth day, and 30 on the thirty-fifth day. Controls were obtained by selecting and similarly processing the following numbers of lice from the normal stock colony: 10 first-instar lice on the first day, 13 second-instar lice on the fourth day, and 30 adults (14 males, 16 females) several days after the thirty-fifth day.

Serial sections of 19 first-instar lice prepared 1 day after infection were examined.

Of these lice, 17 showed *P. tularensis* exclusively within the lumen of the midgut (Pl. XVIII, fig. 1). These organisms were found to range all the way from the caeca to the portion of the digestive tract approaching the rectal papillae. Often they were noted to be more concentrated toward the most posterior portion of the midgut. Where the gut content clouded the observation of the major portion of the lumen, organisms usually could be seen near the periphery of the lumen adjacent to the epithelial cells.

Of the 21 lice examined from the group fixed 4 days after infection, 16 were infected with *P. tularensis*. All 16 of these revealed organisms present in the lumen of the midgut. Four of these 16 lice also showed some degree of intracellular infection, this infection being very light and limited to one or several cells in the region of the juncture of the oesophagus and the midgut; these cells were dorsal in position and occurred in the groups of cells adjacent to the entering oesophagus (Pl. XVIII, fig. 2). The lack of distension of the infected cells and the paucity of the *P. tularensis* gave a clear picture of the early stages of infection.

Of the 25 lice fixed 10 days after infection, 14 were tularemia infected. Four of these showed organisms restricted to the gut lumen. The 10 remaining lice which demonstrated intracellular infection could be placed in three arbitrary groupings according to the degree of infection which they showed. Four revealed only a very limited infection of one to a few cells, dorsal in position and adjacent to the previously mentioned oesophageal juncture with the midgut. They presented a picture quite similar to that of the few lice found infected intracellularly after 4 days.

Four other lice showed a more extensive infection which, however, did not extend beyond the bounds of the gut epithelium. The infection at the oesophageal juncture involved more cells and showed greater distension of those cells involved. Besides this infected region, other areas of the midgut became involved occasionally. Infected single cells or groups of cells extended forward into the caeca (Pl. XVIII, fig. 3) and as far back as half way through the midgut.

The two remaining lice demonstrated not only an extensive infection of the midgut epithelium but also an invasion of their body cavity by *P. tularensis*. Observation of the widespread infection and the accompanying distortion and obliteration of the infected cells made the source of entrance into the haemocoele fairly clear. In one louse, with a very heavy infection spreading anteriorly and posteriorly from the oesophageal juncture, an area of the caecal wall was noted where the organisms apparently had broken through into the haemocoele. Although no such specific point of entry was established for the other louse, the heavy infection and cell destruction in the oesophageal-juncture region probably provided the site of entry to the haemocoele. The latter louse was unusual in showing an extensive infection of the midgut epithelium toward the posterior portion of the gut. This was the only instance of such an infection among all lice examined in the experiment.

The *P. tularensis* free in the haemocoele appeared to be dispersed evenly throughout the haemolymph. The bacteria were found lying between muscles, fat bodies, reproductive organs, and other internal structures. An occasional blood cell was encountered that appeared to contain a number of the organisms in its cytoplasm.

Such presumably phagocytic cells were more conspicuous toward the posterior of the abdomen.

Of the 27 lice taken from the colony 17 days after infection, only 10 (five males, five females) showed the presence of *P. tularensis*. No differences in infection due to sex were apparent. Seven lice had tularemia organisms visible in the gut lumen and two had organisms present in the haemocoele. As with the 10-day lice, several (3) had a very light infection near the oesophageal-midgut junction, others (5) showed a much more extensive infection of the midgut epithelium, while in the remainder (2) the haemocoele as well as the gut cells were involved. The infection of all of these predominated in the region of the dorsal cells around or near the junction of the oesophagus to the midgut. In the more heavily infected lice the infection spread both forward into the caeca and posteriorly farther into the midgut, but no infected cells were seen in any lice beyond the anterior third of the midgut.

An examination of the serial sections of 30 lice (15 males, 15 females) killed 35 days after infection revealed only three infected lice (two males, one female). All three showed numerous organisms in the gut lumen, and although only one displayed the presence of *P. tularensis* in the body cavity, they all exhibited such heavy infection of the midgut epithelial cells that haemocoele invasion was presumably imminent (Pl. XVIII, fig. 4).

Table 1 summarizes the data presented above. A comparison of the percentages of lice in the various stages of infection shows that, with the passage of time after infection, there is a tendency for the *P. tularensis* to shift from the lumen of the gut to the gut epithelium and eventually to the body cavity.

Table 1. *Progress of tularemia infection in lice*

Days after infection	Total lice examined	Percentage of lice infected	Percentage of infected lice with: Lumen infection only	Light midgut infection	Heavy midgut infection	Heavy midgut and haemocoele infection
1	19	89	100	0	0	0
4	21	76	75	25	0	0
10	25	56	29	29	29	13
17	27	37	0	30	50	20
35	30	10	0	0	67	33

The infection of the epithelial cells of the midgut occurred predominantly in its anterior portion. To determine how prevalent this tendency was, a tabulation of the percentages of lice showing cellular infection of the various regions of the gut was made. The numbers used in the designation of the gut regions infected were a series of landmarks proceeding posteriorly from the anterior margin of the thorax (0). Subsequent numbers refer to the beginning of the gastric caeca (1), the juncture of the oesophagus and the midgut (2), the first (3), second (4), third (5), and fourth (6) abdominal spiracles, the anterior portion of the recurved midgut (7), the most posterior portion of the midgut (8), and the end of the abdomen (9). A total of 44 lice with intracellular infection of the midgut obtained from several

experiments was employed. The percentages of lice infected in the particular gut regions are as follows:

Regions of gut								
0–1	1–2	2–3	3–4	4–5	5–6	6–7	7–8	8–9
0	66	98	36	16	2	2	5	0

It is evident from this that infection of the anterior third of the midgut predominated among the infected lice and only rarely was infection noted in the posterior two-thirds.

As would be expected, cells in all stages of infection were found. Cells in the early stages of infection showed very little if any distension and appeared normal in shape. However, advanced infection gave a typical picture of cellular swelling or ballooning (Pl. XVIII, fig. 3). The tularemia organisms uniformly filled the entire cytoplasm of the cell, causing the cell to become distended and to protrude into the lumen of the gut. The consistent occurrence of intact nuclei made it appear that the *P. tularensis* did not invade these but multiplied only in the cytoplasm of the infected epithelial cells. Occasionally an infected epithelial cell was noted free in the gut lumen. Rarely cells were observed, which seemed to have burst, with their walls bordering on the lumen apparently having ruptured and released their contents.

An examination of the control slides showed no organisms, either intra- or extracellular, that could be confused with the *P. tularensis* observed in the infected lice.

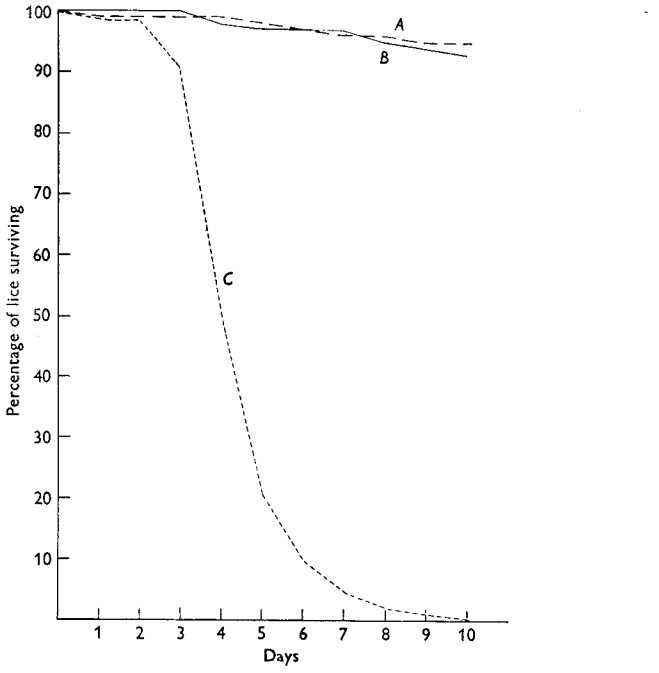
INOCULATION OF THE LOUSE HAEMOCOELE

With the observation in several instances of *P. tularensis* in the haemocoele of the louse, it became of interest to determine what effect, if any, the presence of the organisms in this cavity had on the longevity of the louse. The inoculation of tularemia organisms directly into the haemocoele offered an approach to this problem. The lice used for the body cavity inoculation of *P. tularensis* all were within their first 2–4 days of adulthood. They were inoculated with a fine pin inserted near the lateral margin of the ventral integument at the level of the fifth abdominal segment. This pin was dipped into a 24 hr. GCBA culture of the tularemia organisms immediately before each abdominal puncture. A simple and rapid insertion and withdrawal of the pin was made in the hope of avoiding as much damage to internal structures as possible. After inoculation, the lice were stored at 28° C. and 60% relative humidity, and were fed once daily on normal rabbits. In this manner 150 lice were inoculated with *P. tularensis*.

Two control groups of lice were maintained along with this infected group. One control consisted of 108 lice which had been punctured with a sterile pin in exactly the same manner as the above infected group. The second control group contained 152 lice which had undergone no special treatment. Both control groups were maintained under the same conditions as the infected lice. At each feeding a count of both dead and surviving lice was made; in this manner every louse was accounted for. The criterion for dead lice was established to include not only those lice which

were actually dead but also those which were so near death that only a very weak peristalsis or an occasional leg tremor was present.

For the first 2 days after the inoculation of the lice, all three groups showed similar survival. However, the death of 11 lice in the infected group on the third day heralded the onset of a rapidly progressing mortality within that group. Sixty-one deaths occurred on the fourth day, 45 on the fifth day, and excessive mortality continued until all lice were dead on the tenth day. Both control groups showed similar and almost perfect survivorship. Text-fig. 1 illustrates the findings of this experiment.



Text-fig. 1. Survivorship of lice after body cavity inoculation with *Pasteurella tularensis*. The lines represent (A) normal lice, not punctured, (B) normal lice, punctured, and (C) infected lice.

In order to confirm statistically the difference in survivorship between the infected and control groups, the percentages were transformed to logits and the days to logarithms, since these two metameters appeared to approximate a linear relation most clearly among the several transformations investigated. Straight lines were fitted separately to each group by the method of least squares. An analysis of variance as shown by Bliss & Calhoun (1954, p. 133) was used to determine significant differences among the slopes of the three lines. Heterogeneity among the three slopes was demonstrated by an F value of 37·6 ($P \ll 0{\cdot}001$). The

slopes of the two control groups did not vary significantly from each other ($F = 1{\cdot}2$, $P > 0{\cdot}05$), while each of the control slopes differed significantly from the slope of the infected group at $P < 0{\cdot}01$. These findings clearly confirm the fatal effect of a haemocoele infection of *P. tularensis* in the louse.

Plate counts made of 10 lice on the initial day of infection resulted in maximum readings of 4000, minimum of 90, and an average of 1300 organisms per louse. In 10 lice tested on the fourth day after infection, the maximum was 90×10^6 organisms per louse, the minimum was 13×10^5 organisms, and the average was 36×10^6. Only five lice were used for plate counts on the seventh day after infection, but these showed even more extensive multiplication of the tularemia organisms, the maximum and minimum being 14×10^7 and 5×10^7, respectively, the average being 11×10^7 organisms per louse. To preserve the numbers of the infected colony from depletion by these counts, the 10 lice for the initial day were extra lice above the 150 originally inoculated. The lice on the other 2 days were moribund. No healthy lice were ever removed from the infected colony for this purpose.

Various means besides plate counts were used to demonstrate the infection in the lice. Crude platings of undilute suspensions were made of seven dead or moribund lice on the third day and five such lice on the eighth day. All 12 lice showed confluent growth of *P. tularensis* on the GCBA plates. A Gram stain of smears of body fluids from 10 lice dead on the fourth day showed abundant typical *P. tularensis*.

Serial sections were made of 10 lice dead on the fourth day and of 3 lice, the last members of the colony, on the ninth and tenth days. All 13 lice showed *P. tularensis* scattered throughout the haemocoele. The older lice appeared to have a heavier infection of the body cavity. Although the organisms were predominantly extracellular, occasionally intracellular *P. tularensis* was observed in various tissues of virtually all of these lice. Sporadic infection of the epidermal cells of the oesophagus, tracheae, body wall, and vaginal wall were observed, along with an additional infection of longitudinal and dorso-ventral muscles, and of the accessory gland in one louse. This last case might be attributed to the penetration of the organ by the inoculating pin. Seven of the lice showed infected epidermal cells surrounding the original pin prick through the body wall. The organisms showed abundant intracytoplasmic development similar to that previously described for the midgut epithelial cells. The cells were usually quite swollen and fully packed with *P. tularensis*. Infected areas typically involved only isolated groups of a few cells. An occasional phagocytic blood cell was seen to contain a number of *P. tularensis*. The small number of organisms in these cells would indicate absence of active intracellular multiplication of the bacteria.

Of the original 150 lice in the infected colony, 50 were examined by one means or another and were all found to harbour *P. tularensis*. Serial sections of eight normal lice were inspected in the control group receiving a pin prick but no *P. tularensis*. In none of these lice could tularemia-like organisms be demonstrated.

DISCUSSION

Tularemia infection in the louse. In a previous study on the multiplication of the tularemia organisms in the human body louse (Price, 1956), it was found that lice were initially 100% infected. Following this initial infection, some lice lost their infection and others showed an increase in the number of tularemia organisms. The percentage of uninfected lice increased with passage of time after initial infection until only a very small percentage retained the infection after 35 days.

In an attempt to explain this phenomenon within an infected louse colony, it is necessary to emphasize certain points concerning the infected louse. Histological study revealed *P. tularensis* limited to the lumen of the midgut of some lice as long as 10 days after infection. Apparently the first manifestation of intracellular development of *P. tularensis* may occur as early as the fourth day after infection or as late as the seventeenth day or even later. As long as the infection was limited to the gut, the louse probably was not injured by the infection, but once the *P. tularensis* penetrated the barrier of the gut wall into the haemocoele, death occurred within a week.

It seems unlikely that a louse may be capable of losing its infection once it has become intracellularly infected. With the passage of time after initial infection the percentage of infected lice showing heavy midgut epithelium and haemocoele involvement becomes progressively larger. Were lice becoming uninfected from a heavily infected level, lice exhibiting lower levels of infectivity would be present at this interval after infection. An examination of Table 1 reveals that such is not the case. The high susceptibility of the body louse to *P. tularensis* in the body cavity lends added support to the belief that heavy louse infection ends in death. Heavy epithelial infection was invariably accompanied by cellular distortion and destruction that made the break-through of the bacteria into the body cavity seem imminent. Since the louse lacks any nidi in its midgut, the replacement of these destroyed cells would not be possible (Sikora, 1916). This progressive picture of the infection in infected lice makes it likely that it was the combination of death of infected lice and disinfection by ridding the gut lumen of the organisms that caused the decreasing percentage of infected lice with time after infection. Those lice which on plate counts showed tremendous development of organisms were undoubtedly those in which invasion of the haemocoele had occurred, and failure to detect more of these is attributed to the fact that only healthy-appearing lice were selected for the counts. Lice suffering from this body-cavity type of infection become moribund or abnormal-appearing a day or two before actual death and thus would not be chosen for plate counts. Those infected lice which persisted the longest were those which were perhaps best able to prolong their lumen infection without cellular involvement, and to prolong cellular invasion longest without a breakdown of the gut wall.

The tularemia-infected cell of the body louse closely resembled the infected cells found by Francis (1927) in *Dermacentor andersoni* and *Cimex lectularius*. The tularemia organisms in all three arthropods invaded the haemocoele; however, whereas the principal site of infection in the louse was in the anterior third of the

midgut epithelium, the tick showed infection of the epithelial cells of the rectal sac, lower intestine, intestinal diverticulae, and Malpighian tubules, and the bed-bug showed intracellular invasion only in the posterior portion of the midgut and occasionally in the Malpighian tubules. There was absence of general multiplication in the gut lumen of the ticks but multiplication in the anterior portion of the bed-bug midgut lumen. Salivary glands of all three failed to demonstrate the presence of *P. tularensis*.

Similarities with rickettsial infections. It is interesting to note that, like the rickettsiae reported by Weyer (1954), *P. tularensis* is able to multiply in the louse haemolymph. It should be noted in this connexion that Weyer's findings are unusual since all pathogenic rickettsiae, except for *Rickettsia quintana*, multiply only intracellularly. Growth of many diverse organisms has been established in the body fluids of the louse. The rickettsiae as well as *Bartonella bacilliformis*, *Trypanosoma melophagium*, and the symbiont of the bed-bug have been shown to multiply abundantly there (Weyer, 1953*a*). The relapsing-fever spirochaete and the tularemia organism also must be added to this list. Weyer believed the haemolymph to be a polyvalent substrate enabling these entirely different micro-organisms to thrive luxuriantly. Even the rapid death of the louse caused by *P. tularensis* presents a situation similar to that observed with some of the rickettsiae. The manner in which these organisms caused the death of the lice was obscure, but Weyer (1952) advanced the suggestion that either a toxic substance of the micro-organism or the blocking of the blood cells might be responsible. The latter possibility was suggested by his consistent finding of numerous rickettsiae in the phagocytic blood cells. The absence of heavy infection of the blood cells by *P. tularensis* makes the hypothesis of toxaemia more likely.

The intracellular development of the tularemia organism in some cells of the body wall, tracheae and muscles following body cavity inoculation had its only reported counterpart with *Coxiella burneti* (Weyer, 1953*b*). One instance was reported by Weyer (1950) of development of *Rickettsia mooseri* in the female gonads following probable injury of these during the artificial body cavity inoculation of the louse. The multiplication of *P. tularensis* in both the cells and contents of the accessory gland of a female louse was believed to have occurred in a similar manner.

In the midgut epithelium, the rickettsiae developed only within the cytoplasm of the infected cells. Degenerative evidence was often revealed by ballooning and vacuolization of the cells. No apparent preference was shown for any region of the midgut. Weyer (1953*a*) attributed this special growth ability in the louse stomach to a fundamental character of all true rickettsiae, which can be interpreted as a sign of common origin. *P. tularensis* showed itself capable of invading the midgut epithelium of the louse in a similar manner, although demonstrating a marked preference for the anterior portion of the midgut. The examination of infected lice 35 days after original infection indicated that this relationship in some cases was not too deleterious to the louse involved.

The foregoing comparisons show that infection of the body louse with *P. tularensis* is similar in most respects to that with the rickettsial pathogens. In view of its Gram-negative, pleomorphic, and minute nature, as well as its intimate relationship with arthropod tissues, *P. tularensis* resembles rickettsiae quite closely. The rickettsiae, however, are incapable of cultivation on artificial media, and in this respect differ from the tularemia organism. The demonstration of similarities of the above organisms in louse infection offers further evidence on the close relationship of *P. tularensis* to the rickettsiae.

SUMMARY

1. *P. tularensis* may be abundant in the midgut lumen, the epithelial cells of the anterior third of the midgut, and the haemolymph of the louse. Multiplication is extracellular in the lumen and the haemolymph and intracytoplasmic in the gut cells.

2. The multiplication of the tularemia organisms in the midgut epithelium eventually leads to the disruption of these cells and the break-through of the organisms into the body cavity. The growth of these organisms in the haemocoele results in the death of the louse in 4–7 days.

3. Lice vary greatly in their susceptibility to infection. Some lice show complete resistance to infection; others are capable of retaining the infection essentially for their normal life span, i.e. 35 days; still others apparently succumb to a rapid increase of the organisms in a relatively brief time after infection.

4. The behaviour of *P. tularensis* within the louse presents interesting similarities to infection of lice with the rickettsiae pathogenic to humans.

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EXPLANATION OF PLATE XVIII

Fig. 1. *Pasteurella tularensis* in lumen of the midgut of a louse 1 day after infection. (× 500.)

Fig. 2. Early infection of midgut epithelium 4 days after infection of a louse. (× 500.)

Fig. 3. Ballooning infected cells in the caecum of a louse 10 days after infection. (× 500.)

Fig. 4. *P. tularensis*, both intracellular and in the midgut lumen, in a louse 35 days after infection. (× 500.)

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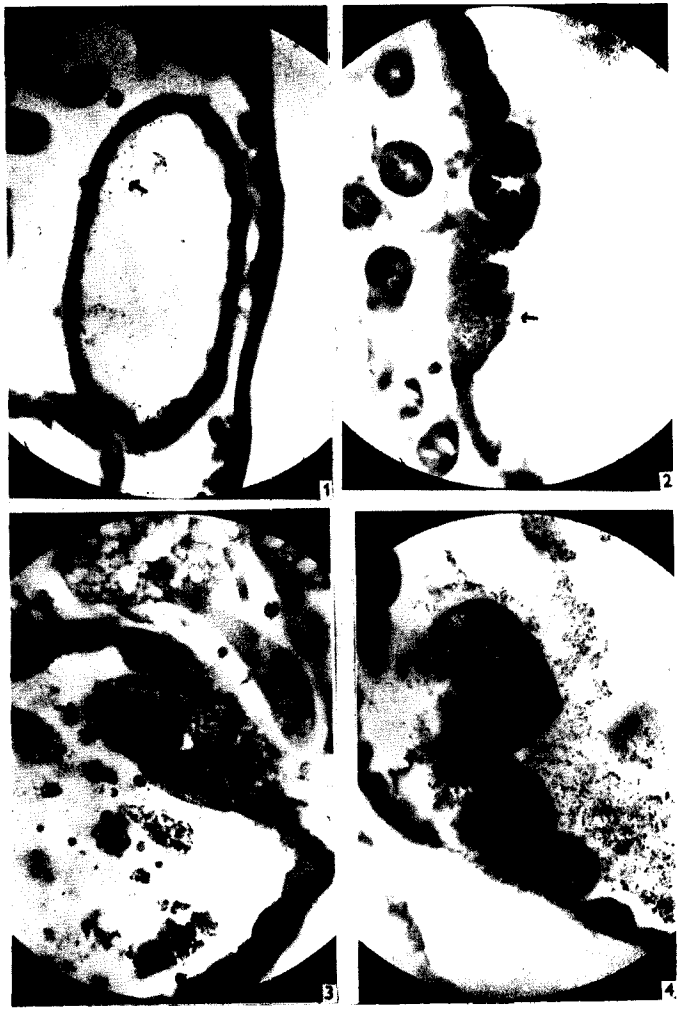

(*Facing p.* 446)