INFECTION AND IMMUNITY, July 1974, p. 257-263

Vol. 10, No. 1
Printed in U.S.A.

Ultrastructural Studies of *Rickettsia prowazeki* From Louse Midgut Cells to Feces: Search For "Dormant" Forms

DAVID J. SILVERMAN, JACK L. BOESE, AND CHARLES L. WISSEMAN, JR.
Department of Microbiology, School of Medicine, University of Maryland at Baltimore, Baltimore, Maryland 21201

Received for publication 5 March 1974

An electron microscope study of infected human louse gut cells and feces was made to determine whether a valid correlation exists between the increased resistance of *Rickettsia prowazeki* (in the louse feces) to adverse environmental influences and changes in the organism which might be reflected in its ultrastructure. Upon fine structural examination of this intracellular parasite as it passed from the louse midgut cell to the feces, it was apparent that no such morphological changes had occurred.

Although usually considered relatively unstable and sensitive to physical and chemical influences, *Rickettsia prowazeki* nevertheless displays remarkable stability at ambient temperatures in dried feces from infected lice (16, 24, 27). This property undoubtedly plays an important role in the spread of typhus fever during epidemics and, prior to the recognition and widespread acceptance of the occurrence and epidemiological significance of recrudescent typhus (Brill-Zinsser disease) (35), it was seriously discussed as a possible means for interepidemic survival of the organism (16, 24, 27).

In the course of other investigations (5), human body lice, *Pediculus humanus humanus* L., infected with the virulent Breinl strain of *R. prowazeki* became available for ultrastructural studies. Accordingly, a search was made of rickettsiae (i) within infected louse midgut cells, (ii) free in the midgut lumen, and (iii) in the excreted feces for possible ultrastructural changes which might be associated with increased resistance to inactivation by adverse environmental conditions.

MATERIALS AND METHODS

Human body lice, *Pediculus humanus humanus* L., were infected with the Breinl strain of *Rickettsia prowazeki* by the membrane feeding technique and were subsequently maintained at 32 C and at a relative humidity of 52%; the lice were then fed daily on a nonimmune rabbit as described previously (5). When the infection neared its peak, louse feces were collected over a 2-h period directly into a 2% glutaraldehyde solution prepared in Millonig phosphate buffer (15) in the same system used previously to examine excreted rickettsiae for antibodies (5).

Infected lice were placed on a glass slide in a drop of fixative. The thorax and abdomen were carefully pulled apart with dissecting needles to expose the intestinal tract. The midgut was removed, transferred to fresh fixative (1% osmic acid in Millonig buffer), and embedded in Epon 812 according to Luft (14). The infected louse feces were processed in a similar manner. Ultrathin sections (60 to 80 nm) were cut on a Porter-Blum MT-2 ultramicrotome with a DuPont diamond knife. The sections were picked up on 300-mesh copper grids and were double stained—first with an aqueous solution of uranyl acetate (0.5%) and then with Reynold lead citrate (19). The sections were examined in a Siemens 101 electron microscope operating at 80 KV which was equipped with a 400-μm condenser and a 50-μm objective aperture.

RESULTS

A typical louse midgut cell which is infected with *Rickettsia prowazeki* is shown in Fig. 1. The gut cell has remained remarkably intact despite the presence of moderate numbers of rickettsiae. Many of the host-cell structures such as the nucleus, mitochondria, microtubules, and rough endoplasmic reticulum appear physically undamaged by the infection. The morphological features of the rickettsiae are typical of those described for gram-negative microorganisms. Rickettsiae having both a smooth and a wrinkled cell wall are seen in this particular section through the louse cell.

Structurally, all of the rickettsiae display a trilaminar cell wall and cytoplasmic membrane, contain ribosomes, and have a dense, fibrous, undefined nuclear region (Fig. 2). Some of the microbial cells possess vacuolar-type inclusions of varying size and electron density, located quite often at the polar regions of the cells.

Surrounding each cell is a relatively large clear area corresponding to the microcapsule whose structure will be described in more detail in a forthcoming manuscript (D. T. Brown, C. L. Wisseman, Jr., and W. T. Walsh, in preparation). In this heavily infected louse gut cell the organelles have been displaced by rickettsiae. The host cell ribosomes are no longer arranged in an orderly array on the rough endoplasmic reticulum, which is now barely visible.

A disrupted louse midgut cell which was observed free in the lumen is shown in Fig. 3. The rickettsiae here appear similar to those within the intact host cell with the exception of a few cells which are plasmolyzed. Host cell mitochondria are once again prominent and appear physically intact.

As the rickettsiae divide and increase in numbers, the host cell enlarges and eventually bursts, releasing many organisms into the lumen of the gut. Figure 4 is an ultrathin section through some rickettsiae which were found free in the lumen. Their structure has not significantly changed from those reckettsiae seen within the midgut cell.

Finally, in Fig. 5 one can see the organisms as they appear in the feces of the louse. All of the features described for the rickettsiae in the louse midgut cell and lumen are identifiable in these cells as well. No new features or observable changes are recognized. Therefore, if there are any changes in the ultrastructure of *R. prowazeki* as it passes from the midgut cell to the feces, they must be very subtle indeed.

DISCUSSION

The unique survival of *R. prowazeki* in the louse vector and its feces might be the result of (i) adaptive changes in the microorganism as it passes from within infected midgut cells, through the gut lumen to the feces, (ii) a physical and chemical environment especially conducive to survival, or (iii) a combination of the two. The present study, although confined to ultrastructural observations, contributes to an understanding of the organism and its changing environment and lends support to the second alternative.

When considering possible adaptive changes in the rickettsia in the louse, the only information available appears to be morphological. Thus, light microscopy has revealed that *R. prowazeki* grows to enormous numbers within cells of the louse midgut, where it often displays the same range of pleomorphism commonly

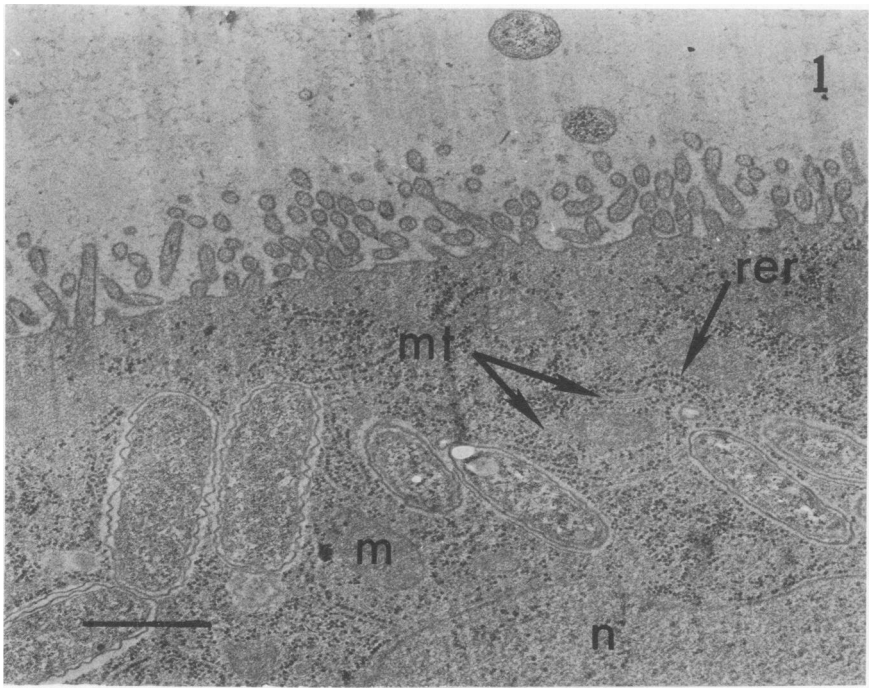

FIG. 1. *Representative ulthrathin section through part of a louse midgut cell (including a portion of the gut lumen) infected with Rickettsia prowazeki. Symbols: n, nucleus; m, mitochondria; mt, microtubules; rer, rough endoplasmic reticulum. Bar, 0.75 μm.*

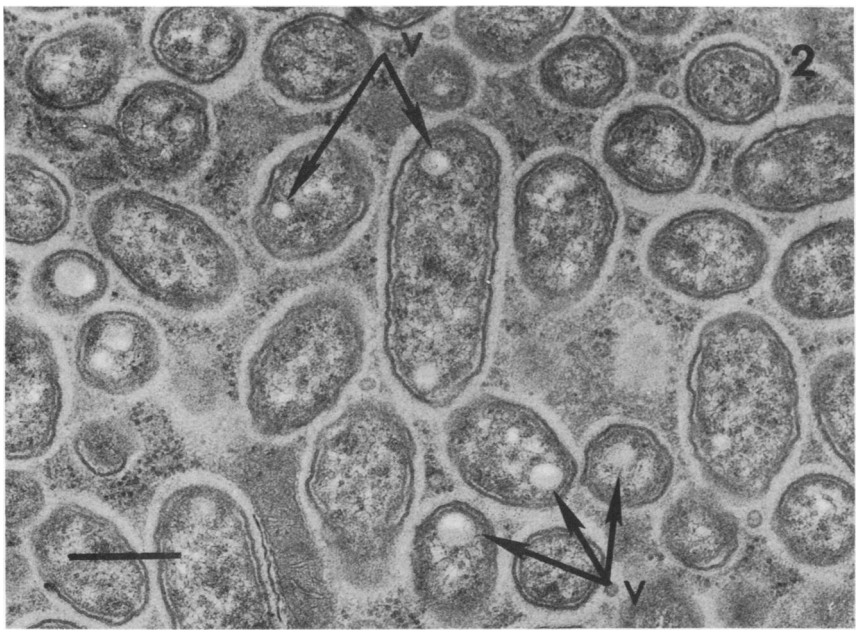

FIG. 2. *Portion of heavily infected louse midgut cell. Note the electron-lucent vacuole-like structures (V) within the rickettsiae, Bar, 0.5 μm.*

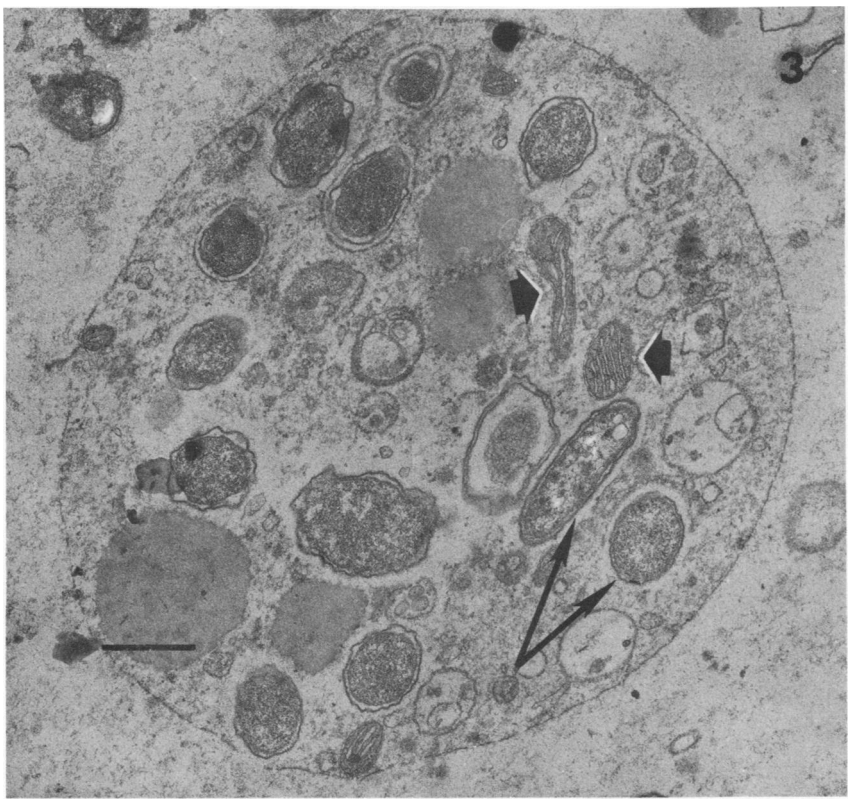

FIG. 3. *Disrupted louse midgut cell found free in the lumen containing many rickettsiae (long arrows) and intact mitochondria (short arrows). Bar, 0.5 μm.*

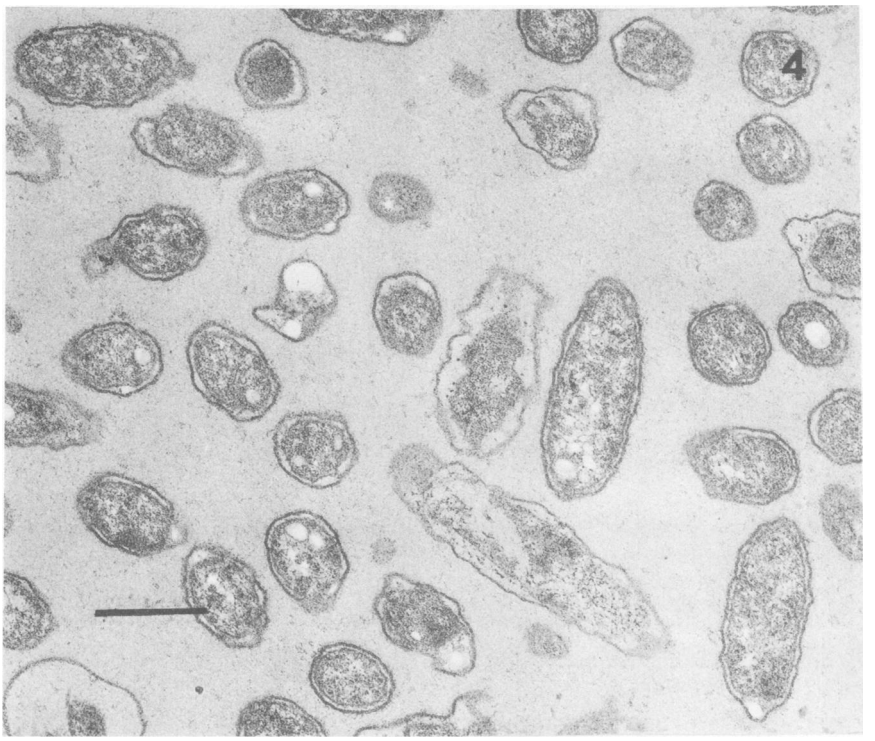

FIG. 4. *Ultrathin section through cells of R. prowazeki typical of those seen in the lumen of the louse midgut. Bar, 0.5 μm.*

seen in other host cell systems, and, as far as is known, retains this variable morphology in the gut lumen and feces (1, 2, 10–12, 20, 33, 34). No unique forms, differing from those seen in other systems, have been described.

The present ultrastructural study failed to demonstrate any basic structural change of the organisms during their passage from the intracellular site in the louse to the extracellular, external site in the feces. Previous electron microscope studies of typhus rickettsiae in louse midgut cells (4, 21–23) are consistent with our observations in this microhabitat. No morphological changes have been observed, other than probable artifacts, in the midgut cells, in the gut lumen, or in the feces which might be interpreted as a shift to "dormant" forms, like those described by Gudima et al. (3, 11) and Kokorin (13) for *R. prowazeki* as well as certain other rickettsiae in other host cell systems. Nor have we observed changes corresponding to the "dense" forms clearly demonstrated by several groups in the case of *Coxiella burneti* (8, 9, 28).

Soviet colleagues (22) have observed frequent empty round "vacuolar" spaces in ultrathin sections of rickettsiae in louse gut cells and we have also observed them here, as well as at all points in the louse system. Their true nature is unknown but they have been seen in other host cell systems (D. T. Brown, C. L. Wisseman, Jr., and W. T. Walsh, manuscript in preparation) and might possibly be interpreted as storage granules.

On the other hand, the range of microhabitats through which the rickettsiae pass, although very great, nevertheless is highly conducive to rickettsial survival. For example, the cytoplasm of the louse midgut cell permits rickettsiae to grow and replicate. Because of the established relationship between host cell organelle structure and function, the ultrastructural examination of infected host cells has much to offer for study of rickettsia-host cell interactions. Thus, rickettsial growth appears to continue after the host cell apparatus for protein synthesis, namely, rough endoplasmic reticulum, has been severely disorganized and probably rendered largely inoperable, a morphological finding consistent in principle with observations that inhibitors of host cell protein synthesis, such as cycloheximide, do not interfere appreciably with rickettsial growth or synthesis (25). On the other hand, morphologically intact but functionally untested mitochondria have been ob-

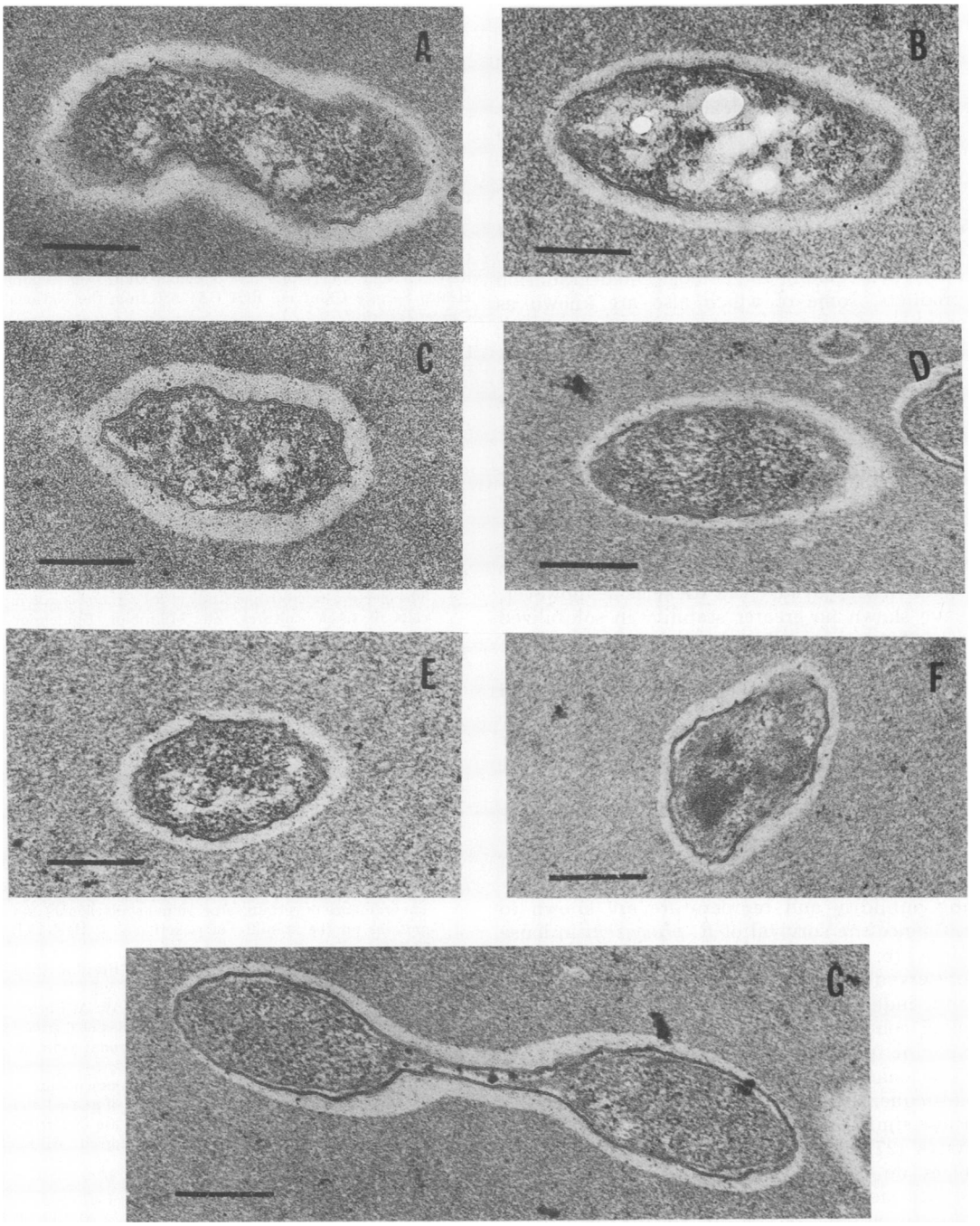

FIG. 5. *Composite of R. prowazeki cells observed in the feces of infected lice. Bar, 0.25 μm.*

served both by us and others (21). Although this observation sparks the imagination, the possibility that typhus rickettsiae might be "adenosine 5′-triphosphate parasites" remains to be proven.

Next, the gut lumen presents to liberated, extracellular rickettsiae a totally different environment which probably does not support growth but nevertheless permits survival. Our ultrastructural studies of gut contents, exclu-

sive of rickettsiae, have been unrewarding. However, when one collects the sparse and little-known biochemical properties of louse gut contents, one finds a remarkable concordance with known physiological requirements of typhus rickettsiae. For instance, the single report uncovered on pH in the louse midgut yielded a value (pH 7.2) which is within the favorable range for rickettsiae (18). Moreover, as a result of proteolytic digestion of blood, the midgut undoubtedly contains proteins, peptides, amino acids, hemoglobin, and hemoglobin degradation products, some of which also are known as stabilizing agents or as substrates for oxidative metabolism for typhus rickettsiae (5–7, 29–31). Viable typhus rickettsiae withstand at least brief exposure to certain proteolytic enzymes (26, 32). Finally, although the hemin requirements of typhus rickettsiae are not known, *Rochalimaea quintana*, an extracellular parasite in the louse midgut, seems to have a nutritional requirement for this blood derivative (17). Thus, the contents of the midgut seem to provide conditions and substances known to foster rickettsial survival. Rickettsiae, however, have shown no greater stability in solubilized louse feces than in blood or tissues (27).

Various secretory and excretory products, such as uric acid, are added in the midgut and rectum (29). Then, in the rectum, water is efficiently extracted by the rectal glands or papillae (30), causing rapid desiccation of the rickettsiae while embedding them in a protective milieu. Feces are dejected as minute highly desiccated particles (30; Charles L. Wisseman, personal observation) and subjected to further rapid drying in the air. The subsequent prevailing humidity and temperature are known to influence the survival of *R. prowazeki* in louse feces (16, 24, 27). Typhus rickettsiae are easily preserved in the laboratory by freeze-drying and, indeed, have effectively survived far less ideal conditions of desiccation in the dust in vaccine production laboratories. Moreover, rickettsiae added to a solution of louse feces and subsequently air-dried survived no better than those similarly dried in blood of tissue homogenates (27). These findings suggest that louse feces do not contain unique protective substances not found in other biological materials.

In the absence of morphologically identifiable changes in the organism, we are led to the conclusion from the available evidence that the chemical and physical environment in the louse feces, although not necessarily unique, probably contributes substantially to the long survival of typhus rickettsiae in louse feces. However, the possibility of adaptive changes of a physiological nature in the rickettsiae, unaccompanied by morphological changes detectable by electron microscopy, has not been excluded as also contributing to this phenomenon.

ACKNOWLEDGMENTS

We gratefully acknowledge the assistance of William T. Walsh in the infection of the lice and initial fixation of the louse guts and of Dennis T. Brown for embedding the specimens.

This study received partial support from Research Contract DA-DA-17-71-C-1007 with the U. S. Army Medical Research and Development Command and from Public Health Service Grant no. RO1 CA15085 from the National Cancer Institute.

J. L. B. is a postdoctoral fellow in rickettsiology under Public Health Service Training Grant no. PHS AI 00016 from the National Institute of Allergy and Infectious Diseases.

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