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Host-Parasite Interaction and Development of Infraforms in Chicken Embryos Infected with *Coxiella burnetii* via the Yolk Sac

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Two phase I strains of *Coxiella burnetii* of different virulence were injected into the yolk sacs of chicken embryos, and the yolk sacs and livers were examined at intervals by light, fluorescent, and electron microscopy. The high absorptive and digestive capacities of the yolk endoderm contributed to the entrance of the organisms into endodermal epithelial cells where *C. burnetii* multiplied. Organisms multiplied not only inside specific vacuoles originating from phagolysosomes but also in the cytoplasm itself. Lysis of the limiting membrane of some phagolysosomes, a normal function of endodermal cells, as well as rupture of vacuoles, provided the release of *C. burnetii* into the cytoplasm. The *C. burnetii* strain of greater virulence infected 100% of the endodermal cells, whereas the strain of lesser virulence infected only 60%. Budding of very small particles from the *C. burnetii* bodies was demonstrated. The particles were regarded as filterable forms of the organism. Despite the enormous multiplication of *C. burnetii* in the endodermal cells, organisms were only rarely detected in the vitelline blood vessels and liver sinusoids of the embryos. Peculiarities of the infectious process of *C. burnetii* in chicken embryos and possible mechanisms of limitation of spread of the infection are discussed.

The ability of *Coxiella burnetii* to multiply extensively in the yolk sac of chicken embryos has been recognized since the time of discovery of the organism (10, 11). It is known also that the infectious processes caused by *C. burnetii* chicken embryos are different from the infectious processes in laboratory animals (Table 1). However, much less is known of the details of host-parasite interaction in the yolk sac which might explain the peculiarities of the infectious process in chicken embryos.

In chick endodermal cells cultivated in vitro, *C. burnetii* multiplies inside large vacuoles without marked damage to the cells (28, 29). This mode of multiplication is also observed in in vivo animal cells and in cell cultures of animal origin (8, 32). However, we have no morphological data on the behavior of *C. burnetii* toward endodermal cells of the intact developing yolk sac. As a rule, generalized infection of the embryo after introduction of *C. burnetii* into the yolk sac has not been observed (22, 25). Leyck and Scheffler (21) found organisms in print preparations of various organs of infected embryos, but did not show that *C. burnetii* was located intracellularly.

Infraforms of *C. burnetii* can pass through collodion membranes with a mean porosity of 132 to 140 nm and remain unsedimentable at 100,000 × *g* for 18 h (19).

The biological significance of filterable forms is not entirely understood since the mechanism of the formation has not been studied. Current conceptions of *C. burnetii* morphology are based on examination of infected cells and tissues (3, 12-14) and purified suspensions of organisms (9, 24, 30). Only Anacker et al. (3) and recently McCaul et al. (T. F. McCaul, T. Hackstadt, and J. C. Williams, *in* W. Burgdorfer and R. L. Anacker, ed., *Rickettsiae and Rickettsial Diseases,* in press) mentioned the existence of very small organisms. Anacker et al. (3) found small particles supposed to be "atypical" organisms in sections of infected chicken embryo yolk sacs. The rickettsial origin of these particles, however, was not proven by binding of labeled antibodies at that time. McCaul et al. (in press) found small particles in purified yolk sac cultures of *C. burnetii.* The particles were formed under the cell wall of the organism and had some of the characteristics of endospores. There is no literature on the connection of *C. burnetii* and its infraforms with the structures of the yolk sac. The aim of this paper is the morphological study of the host-parasite interaction in the epithelial layer of the yolk sac of the developing chicken

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TABLE 1. *Differences between C. burnetii (CB) infection in laboratory animals and in chicken embryos infected into the yolk sac*

Features of infectious processes	Infection in:		Reference(s)
	Animals	Chicken embryos	
Localization of organisms	In mononuclear phagocytes	In epithelial cells (yolk endoderm)	11, 28
Spread	Generalized infection	Limited to yolk sac	25, 22
Formation of infraforms of CB	Inconstantly	Constantly	18, 19
Phase variation	No	Yes	27

embryo, with special reference to the alteration of infected endodermal cells and their relation to the vitelline vessels and to the smallest forms of the organism.

MATERIALS AND METHODS

Organisms. Two phase I *C. burnetii* strains were used: mildly virulent "Apodemus microtii-Lauga" strain (2) (rarely kills infected guinea pigs) and highly virulent "Tabanidae-Kazakhstan" strain (1) (often lethal in infected guinea pigs). Chicken embryos developed for 7 days were challenged by inoculation of one of the *C. burnetii* strains into the yolk sacs and were examined at intervals.

Morphological procedures. The midregion of the area vasculosa was taken from the yolk sacs and fixed in toto with 10% Formalin or 1% osmium tetroxide. Paraffin sections of Formalin-fixed yolk sacs from 15 normal and 30 infected embryos (15 embryos for each strain), as well as semithin araldite sections of OsO_4-fixed yolk sacs from 6 normal and 10 infected embryos, were used for histological study. Paraffin sections were stained with hematoxylin and eosin, using the Giemsa-Romanovsky method, or were processed with fluorescent anticoxiella rabbit immune gamma globulin by the direct Coons method (working dilution of fluorescent conjugate, 1:32). Some of the preparations examined by the direct Coons method were fixed again with acetone, strained by Giemsa, and restudied by using a fluorescent microscope. Semithin araldite sections were stained with thionine.

Ultrathin araldite sections of yolk sacs taken from 6 normal and 10 *C. burnetii*-infected (by 5 sacs infected with each strain) embryos were used for electon microscopy. Sections were examined by using a JEM-7 electron microscope.

For examination of the spread of infection, paraffin sections of the liver of 12 infected embryos, 11 and 13 days postchallenge, were studied by using the direct immunofluorescent method of Coons.

RESULTS

Uninfected yolk sacs. On day 10 of incubation the yolk sacs had a well-developed endoderm. Endodermal cells joined by desmosomes (Fig. 1) contacted the endothelium of vitelline blood vessels or thin basal membrane. Apical cell portions (Fig. 2) formed microvilli, pinocytotic and coated invaginations, vesicles, and phagosomes, pointing to the high absorptive activity of the cell. Endodermal cells were rich in spherical mitochondria and well-developed smooth endoplasmic reticulum. Single vesicles and invaginations of the basal portions of the endodermal cells suggested that besides absorption there was exocytosis toward the mesoderm.

The same yolk fractions which were observed (6) in the whole yolk were observed on the endodermal surface and in the phagosomes of apical cell portions: electron dense lipovitelline spheres, free lipid drops, and vesicular liquid "continuous phase material" (Fig. 2). Absorbed yolk fractions underwent digestion during movement of phagosomes from apical to basal areas of the cell (Fig. 1). The yolk fractions broke into small vesicles or particles of various densities, and simultaneously some of the lipovitelline spheres underwent digestion in toto, resulting in the formation of larger myelin-like laminated bodies. As phagosomes were moving to the basal part of the cell, some of them lost their limiting membrane, and undigested residual dense and laminated bodies were released into the cytoplasm. Residual material was accumulated in the endodermal cells in large amounts during incubation of the enbryos.

The mesoderm of the yolk sac walls showed gradual development of connective tissue rich in fibroblasts and blood vessels at all stages of incubation. Ectodermal cells adhering to connective tissue formed the outer layer of the developing yolk sacs.

Infected yolk sacs. All parts of the yolk sacs maintained their structure. *C. burnetii* was found in the endodermal cells in which organisms were located inside large vacuoles (Fig. 3). Some of the cells were filled with organisms which showed intense specific fluorescence after treatment with fluorescent antibody. A few infected cells were discovered 3 and 6 days postchallenge (Fig. 4). The number of infected cells markedly increased on subsequent days. Yolk sacs infected with *C. burnetii* of the virulent strain Tabanidae-Kazakhstan showed almost 100% affected cells (Fig. 5) at day 13 postchallenge (day 20 of incubation). Organisms of the mildly virulent strain Apodemus microtii-Lauga

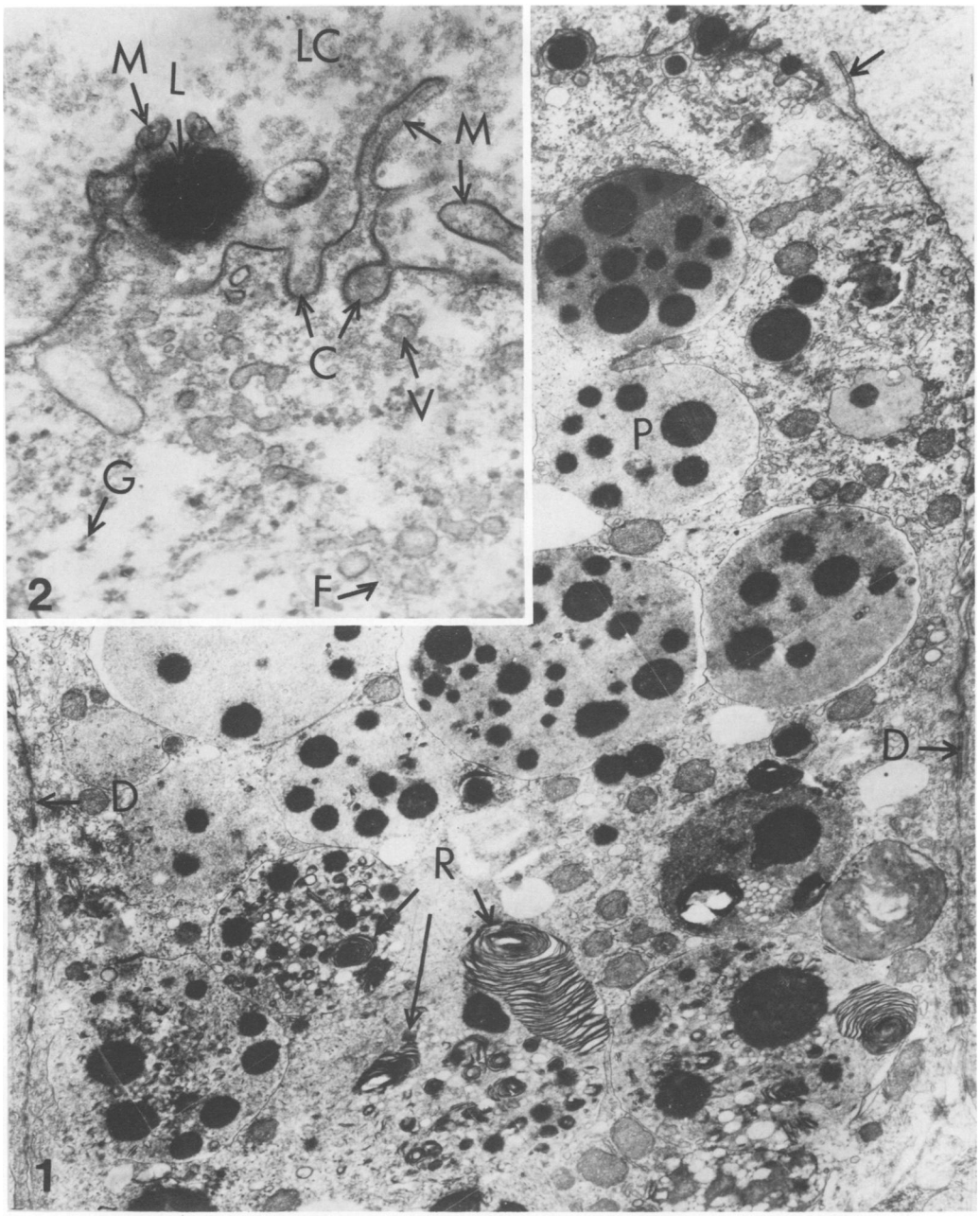

FIG. 1. *The apical and middle portions of the endodermal cells showing microvilli (arrows), desmosomes (D) and connected adjacent cells, and phagolysosomes (P) filled with yolk fractions. The contents of some phagolysosomes of the deeper cell portion are released in the cytoplasm. R, Myelin-like residual bodies. Ten days of incubation. ×5,000.*

FIG. 2. *The apical portion of the endodermal cell showing microvilli (M), coated vesicles and invaginations (C), as well as pinocytotic vesicles (V) involved in the absorption of lipovitelline sphere (L) and liquid "continuous phase" (LC) fractions of yolk. Microfilaments (F) and glycogen granules (G) are shown. Ten days of incubation. ×32,000.*

affected more than 60% of the endodermal cells. Free organisms were discovered outside endodermal cells in the yolks at 13 days postchallenge, suggesting that some infected cells had ruptured. We could not demonstrate the presence of organisms in the mesoderm. Heavily

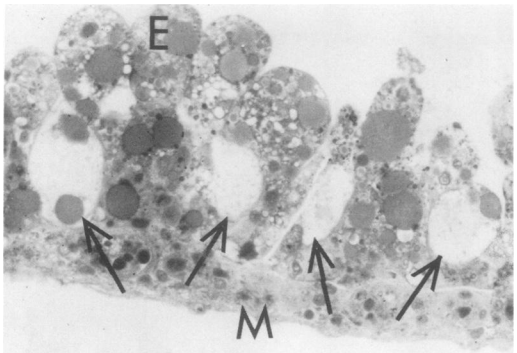

FIG. 3. *Toluidine blue-stained, semi-thin araldite section of the yolk sac showing mesoderm (M) and endodermal cells (E) filled with yolk fractions. Some of the cells contain vacuoles (arrows) with organisms. Eight days postchallenge. ×600.*

infected yolk sacs showed congestion and slight edema at 11 and 13 days postchallenge.

Electron microscope study. Absorption and digestion of yolk by infected endoderm cells were of the same nature as those in the control yolk sacs. Smoothing away of invaginations and microvillous projections of apical plasmalemma of the infected cells, as well as rarefication of the cytoplasm matrix and mitochondrial swelling and dilation of smooth reticulum cysterns, were observed at 11 to 13 days postchallenge. All of these phenomena suggested intracellular edema and diminished uptake of yolk by affected cells at the end of incubation.

Two kinds of *C. burnetii* distribution in endodermal cells were observed: inside the vacuoles and inside the cytoplasm. The latter kind was not detected under light microscopy. Some of the vacuoles containing organisms were indistinguishable from phagosomes. Others were of considerable size, sometimes filling nearly the whole cell (Fig. 6). In addition, at 3 and 6 days postchallenge, colonies of *Coxiella* species were discovered inside large lipovitelline spheres which were undergoing digestion in toto (Fig. 7 and 8).

These organisms formed almost a pure culture in the spheres without admixture of yolk particles. As a rule, the contents of large vacuoles were well separated from the cytoplasm of the cells by membrane units. However, rupture of the vacuolar membrane and invasion of the organisms into the cytoplasm of the infected cells was observed in a few cases (Fig. 9).

In cells that did not contain specific vacuoles, the organisms were distributed in the cytoplasm among phagosomes and cell organoids. At 11 and 13 days postchallenge, infected cells showed swelling with rarefication of the cytoplasm matrix, disappearance of glycogen granules, and microfilaments (Fig. 10).

The two morphological variants of *C. burnetii* previously reported in the literature (9, 24, 30) were observed in the cells infected with *C. burnetii* strains: small, rod-shaped forms with condensed nucleoids and large rounded forms with loose nucleoids. None of the variants showed signs of degeneration. They either underwent binary fission or they divided into two or three unequal parts (Fig. 8). Small, rod-shaped forms were observed predominantly in the denser colonies where organisms seemed to multiply quickly. The large rounded forms prevailed in some of the looser colonies.

The smallest round bodies, 30 to 60 nm, were discovered in the endodermal cells infected with both *C. burnetii* strains. They were hardly distinguished from the yolk particles and smallest laminated bodies scattered among organisms in the vacuoles. When *C. burnetii* multiplied in the cytoplasm, the bodies resembled Golgi and pinocytotic vacuoles. However, the presence of *C. burnetii* colonies inside the lipovitelline spheres in the form of pure culture suggested that these bodies were of rickettsial origin (Fig. 11a) and were formed by the budding of normal-sized organisms (Fig. 11b and c).

Two variants of the smallest bodies could be distinguished: vesicle-like bodies surrounded by one membrane unit (Fig. 11d) and bodies containing a central dense granule resembling a nucleoid (Fig. 11e). Some of the latter group had a double membrane complex. These variants sometimes formed short chains which are like multiplying organisms (Fig. 11f).

Infected endodermal cells maintained the normal direct connection with underlying blood vessels and other structures of the mesoderm. Single organisms were discovered inside vitelline capillaries and veins (Fig. 12). However, no organisms were observed outside of blood vessels in the mesoderm. The mesoderm of the infected yolk sacs did not show alteration of normal structures, although intercellular collections of fluid and dilation of smooth endoplasmic reticulum of mesodermal cells (at 11 to 13 days postchallenge) can be regarded as signs of edema of the yolk sac walls.

The invasion of blood vessels by *C. burnetii* through intercellular spaces of endoderm was not observed. The infected endodermal cells maintained both their epithelial nature and their tight cell-to-cell connections by desmosomes and junctional complexes.

A few organisms were discovered in the liver in only three embryos heavily infected with the virulent strain of *C.burnetii* at 13 days postchallenge. The organisms located inside the liver sinusoids did not form typical colonies.

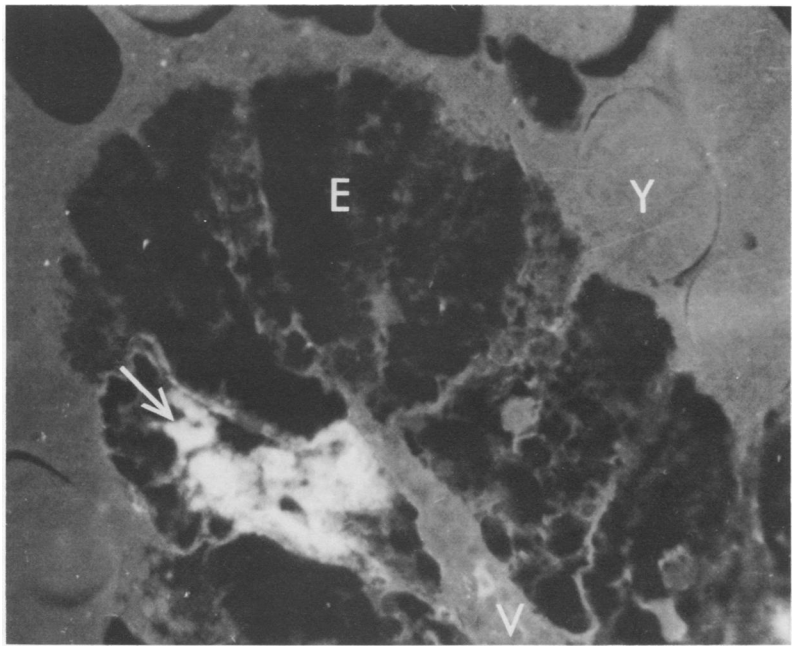

FIG. 4. *Specific fluorescence of the organisms collected (arrow) in a single cell of the fold of the yolk endoderm. Shadows of uninfected endodermal cells (E), unspecific fluorescence of the yolk (Y), and vitelline blood vessels (V) are shown. Paraffin section treated by direct immunofluorescence method. ×800.*

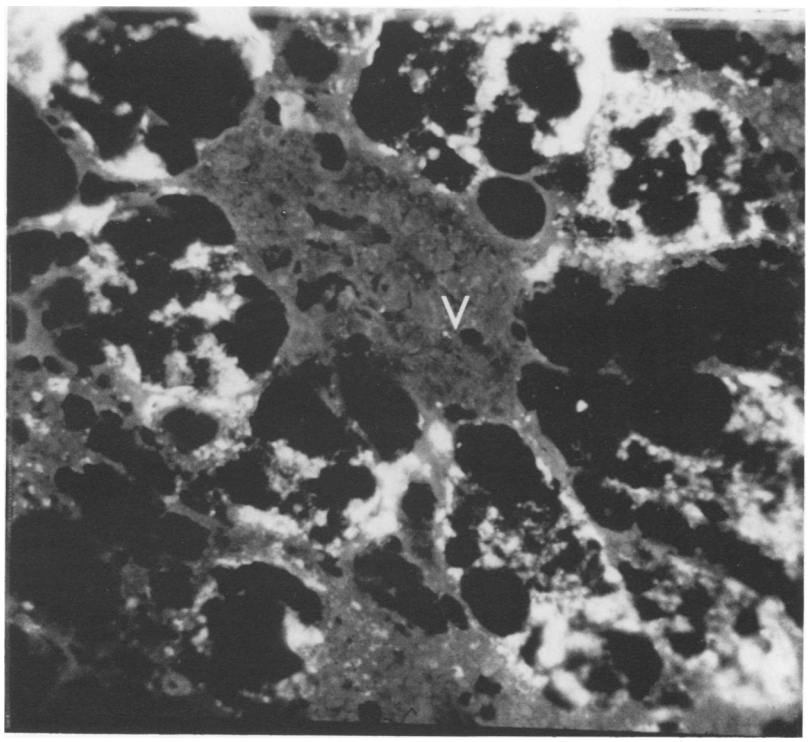

FIG. 5. *Paraffin cross section of the endodermal fold, 13 days postchallenge. All endodermal cells contain collections of organisms showing specific fluorescence. V, Vitelline blood vessel. Direct immunofluorescence. ×800.*

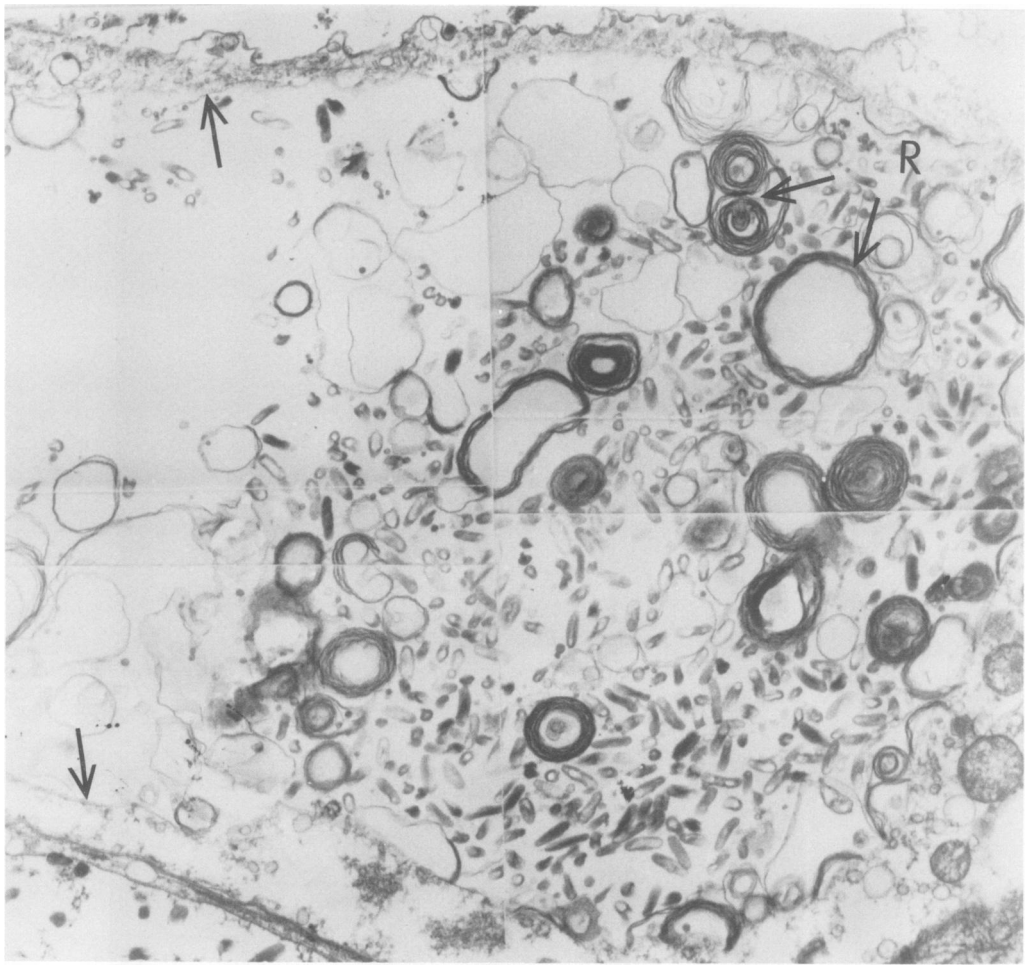

FIG. 6. *Endodermal cell filled with a large vacuole containing a colony of the rod-shaped organisms and myelin-like residual bodies (R). Arrows point to the membrane of the vacuole. Six days postchallenge.* ×5,000.

DISCUSSION

The infectious process caused by *C. burnetii* in chicken embryos inoculated via the yolk sac is influenced by the biological features of the embryos. The yolk endoderm, which is the only site of *C. burnetii* multiplication in this system, displays high absorptive and digestive activities. This had been observed also by other authors (5, 20, 26). As a result, cells of the endodermal epithelium resemble specialized phagocytes and engulf injected organisms.

C. burnetii of the highly virulent strain Tabanidae-Kazakhstan causes more widespread involvement in the infectious process than the mildly virulent strain Apodemus microtii-Lauga. The peculiarities of the infectious process in yolk endoderm caused by *C. burnetii* of both strains are identical. They are connected with *C. burnetii* multiplication in the cytoplasm of the host cell, with limited penetration of blood vessels by organisms and with the constant appearance of infraforms.

The multiplication of *C. burnetii* in cytoplasm is associated with the lysis of the phagolysosome membranes and the membranes of some specific vacuoles containing organisms. The lysis of phagolysosome membranes seems to be normal for endodermal cells (15), and high lysosomal activity of these cells has been reported (15, 26).

The residence of *C. burnetii* inside specific vacuoles of endodermal cells differs from the intravacuolar location of the parasite in animal cells in the following ways. (i) In endodermal cells, the vacuole contains yolk material undergoing digestion (the phenomenon confirms the view that vacuoles originate from phagosomes [4]), and the pathogens multiply directly

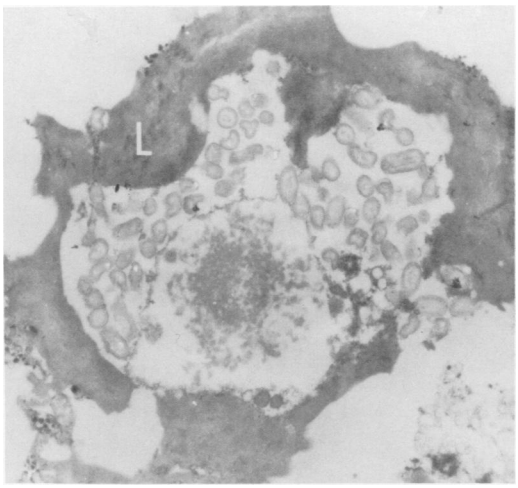

FIG. 7. *Lipovitellin sphere (L) containing a colony of organisms. Three days postchallenge. ×5,000.*

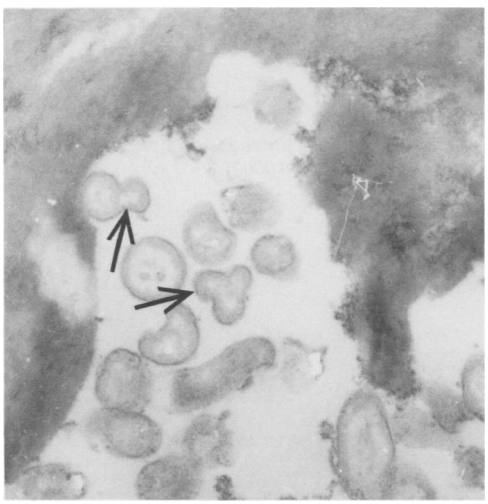

FIG. 8. *Part of the same colony as in Fig. 7 showing uneven division of some organisms (arrows). ×18,000.*

in the yolk particles, showing resistance to lysosomal enzymes. In animal cells, however, the vacuole contains only organisms suspended in the fluid. (ii) Even small specific vacuoles of endodermal cells can rupture, resulting in the release of *C. burnetii* into the cytoplasm without rupture of the host cell.

On the contrary, the vacuole of animal cells is strong, and sometimes displays a two-membrane wall; only giant vacuoles may rupture as a result of overfilling with the fluid which leads to the destruction of the host cell (T. Khavkin and N. Amosenkova, *in* W. Burgdorfer and R. L. Anacker, ed., *Rickettsiae and Rickettsial Diseases,* in press).

The heavily infected endodermal cells showed swelling, intracellular edema, and decrease of absorption activity. This cannot be explained entirely by local damage from the organisms, because the intracellular swelling coincides with edema of the entire yolk sac. Instead, the phenomenon can be attributed to the general toxic action of *C. burnetii* (22) and to interference of the flow of nutritive material from the yolk sac to the embryo.

Despite the enormous multiplication of *C. burnetii* in the endodermal cells, only occasional organisms can be seen in the vitelline blood vessels which are in intimate contact with the yolk endoderm. Only rarely are organisms found in the liver sinusoids of the embryo. This suggests the existence of inhibitory mechanisms which prevent the spread of infection. It is not clear whether the prevention is primarily associated with selective retention of absorbed material by endodermal cells or with inhibitory mechanisms of the embryo itself. The selective transport of various substances across the yolk sac endoderm is normal in mammals (23, 31). The variability of the forms of yolk absorption by endodermal cells, involving phagocytosis, pinocytosis, and micropinocytosis, suggests that selective transport of yolk fractions (and their maintenance in the cell) is also normal in the chick endoderm.

The lysis of the phagolysosome membrane (which provides the release of *C. burnetii* into the cytoplasm) can be regarded as evidence of selective transport. On the other hand, that the chicken embryo inhibits the development of many organisms (7) suggests that some inhibitory mechanisms prevent colonization of the cells of the embryo itself by circulating *C. burnetii* cells.

Two main morphological variants of *C. burnetii* were observed in endodermal cells. They are referred to by other authors as well and have been the subject of some controversy (17, 18, 30). The prevalance of certain *C. burnetii* forms capable of multiplication in different colonies suggests that the presence of small, rod-shaped and large oval forms reflects maturation rather than degeneration.

Besides the two typical forms of organisms, very small (30-nm) formations were observed. Some of them resemble the bodies observed by Anacker at el. (3) during similar examinations. These bodies are difficult to distinguish from Golgi and pinocytotic vesicles and vesicle-like particles of the yolk. However, the presence of *C. burnetii* colonies inside the lipovitelline spheres in the form of pure culture was convincing evidence that these bodies were of rickettsial origin. They originate from the normal-sized

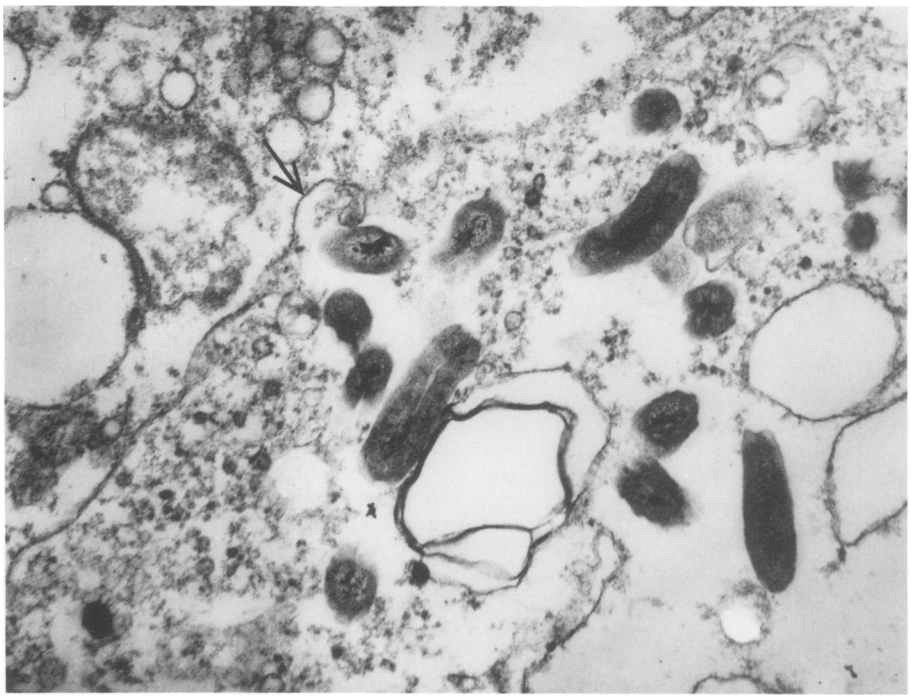

FIG. 9. *Part of a large vacuole containing organisms dispersed among yolk particles and residual material. The membrane of the vacuole (arrow) is ruptured. Six days postchallenge. ×30,000.*

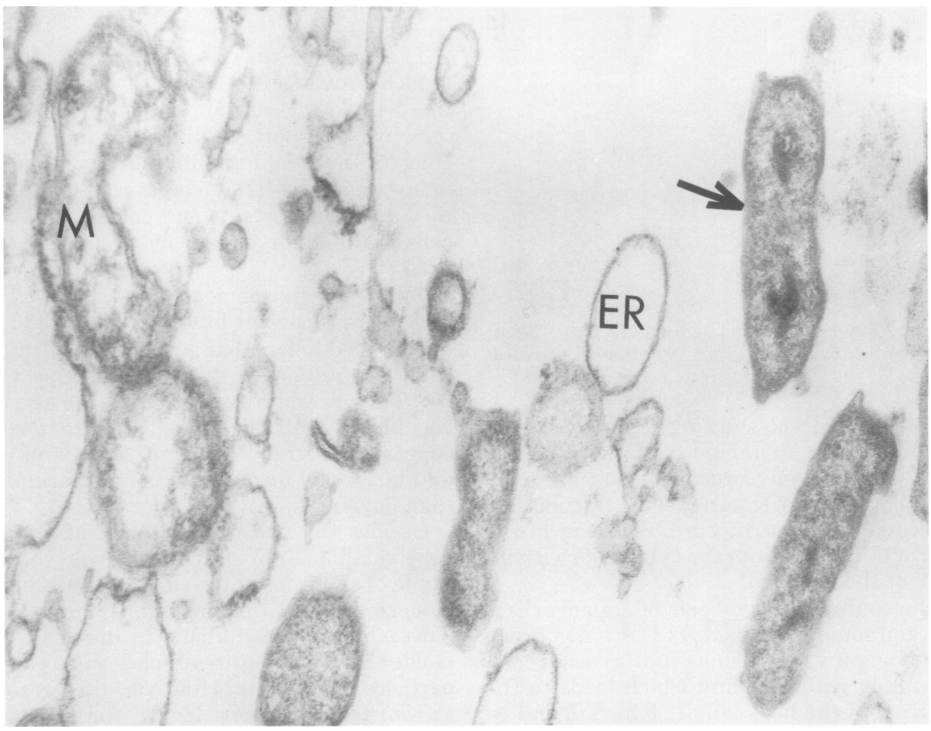

FIG. 10. *Part of infected endodermal cell showing rarefication of the cytoplasm matrix and swelling of mitochondria (M). Profiles of the smooth endoplasmic reticulum (ER) are dilated. Organisms are dispersed in the cytoplasm. One of them (arrow) shows binary fission. Thirteen days postchallenge. ×45,000.*

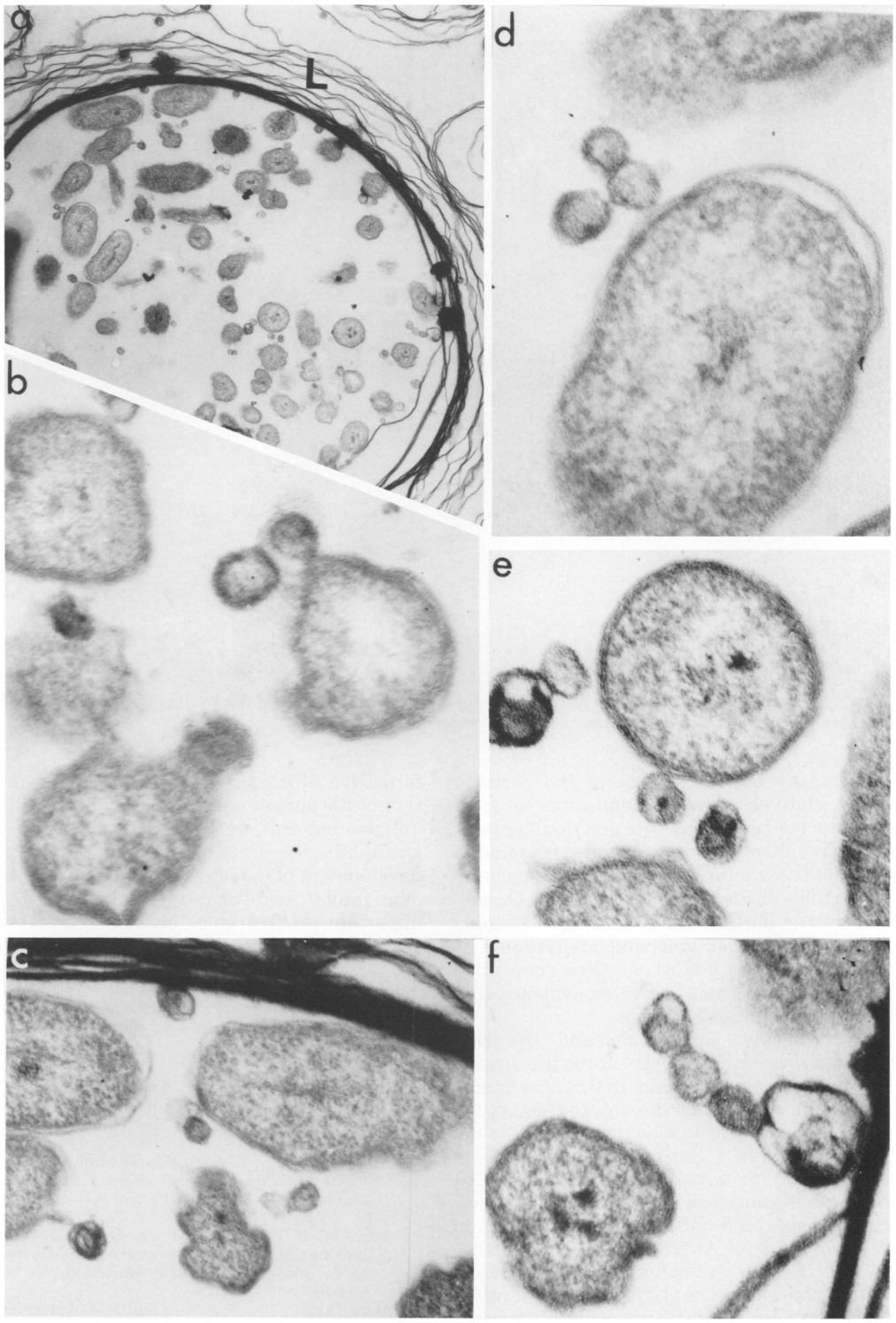

FIG. 11. *The colony of organisms enclosed in the lipovitelline sphere (L) which undergo digestion in toto. (a) View of the whole colony, ×5,000; (b to f) details of (a) showing smallest bodies, their formation by budding from the organisms of normal size (b, c), free vesicular bodies (d), body with central granule (e), and chain of bodies (f). ×60,000.*

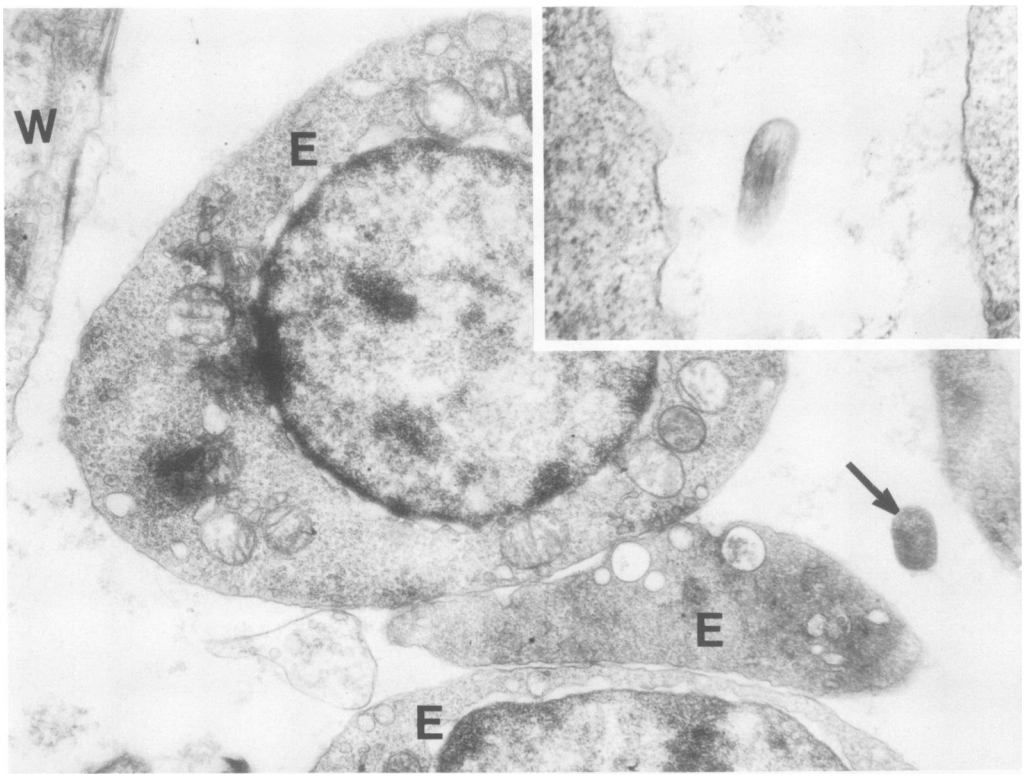

FIG. 12. *Organism (arrow) inside vitelline vein among erythrocytes (E). (W) Wall of the vessel. ×80,000. In the insert, the structure of the organism is shown more clearly. ×15,000.*

organisms by budding. Some of the particles have a relatively simple composition, whereas others have a central granule and double membrane. The phenomenon could be considered to be a sign of the maturation of the inner structure of the bodies during their development. Organization of the particles in short chains suggests the possibility of their independent division. The size of the smallest bodies and their connection with typical *C. burnetii* serve as evidence that they represent filterable forms.

The antigenic composition and the part played by these infraparticles in the life-span of *C. burnetii* remain uncertain. In this connection, it should be noted that the chicken embryo is the only known host in which antigenically deficient variants of *C. burnetii* have been demonstrated (27). It is possible that the smallest forms are also antigenically deficient and therefore could not bind antibody-labeled ferritin in the experiments of Anacker et al. (3). We have observed that the antigen-deficient *C. burnetii* strain of phase II did not bind fluorescent rabbit gamma globulin against organisms of phase II until they were converted into phase I by means of serial passages in animals (16). Therefore, the formation of infraforms of *C. burnetii,* prevention of the spread of infection, and formation of antigen-deficient organisms can reflect the general inhibitory effect of chicken embryo on the development of the organism (7). The possibility of an inhibitory mechanism by the embryo is an important problem to be investigated.

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LITERATURE CITED

1. **Amanzhulov, S. A., N. I. Amosenkova, and O. A. Postricheva.** 1965. On the presence of *Rickettsia burnetii* in *Tabanus straegery.* Med. Parasitol. **34**:612–614. (In Russian.)
2. **Amosenkova, N. I., A. B. Dayter, and K. I. Klenov.** 1959. Investigation of small mammalia in the Lauga focus of Q-fever. Proc. Inst. Pasteur (Leningrad) **20**:71–79.
3. **Anacker, R. L., K. Fukushi, E. G. Pickens, and B. Lackman.** 1964. Electron microscopic observations of the development of *Coxiella burnetii* in yolk sacs. J. Bacteriol. **88**:1130–1138.
4. **Ariel, B. M., T. N. Khavkin, and N. I. Amosenkova.** 1973. Interaction between *Coxiella burnetii* and the cells in experimental Q-rickettsiosis. Histologic and electron microscope studies. Pathol. Microbiol. (Basel)

39:412-423.
5. **Bellairs, R.** 1963. Differentiation of the yolk sac of the chick studied by electron microscopy. J. Embryol. Exp. Morphol. **11**:210-225.
6. **Bellairs, R., M. Backhouse, and R. J. Evans.** 1972. A correlated chemical and morphological study of egg yolk and its constituents. Micron **3**:328-346.
7. **Board, R. G., and R. Fuller.** 1974. Non-specific antimicrobial defences of the avian egg, embryo and neonate. Biol. Rev. **49**:15-49.
8. **Burton, P. R., N. Kordova, and D. Paretsky.** 1971. Electron microscopic studies of the rickettsia *Coxiella burnetii*: entry, lysosomal response, and fate of rickettsial DNA in L-cells. Can. J. Microbiol. **17**:143-150.
9. **Canonico, P. G., M. J. Van Zweiten, and W. A. Christmas.** 1972. Purification of large quantities of *Coxiella burnetii* by density gradient centrifugation. Appl. Microbiol. **23**:1015-1022.
10. **Cox, H. R.** 1938. Use of yolk sac of developing chick embryo as medium for growing rickettsiae of Rocky Mountain spotted fever and of typhus groups. Public Health Rep. **53**:2241-2247.
11. **Cox, H. R., and E. J. Bell.** 1939. The cultivation of *Rickettsia diaporica* in tissue culture and in the tissues of developing chick embryos. Public Health Rep. **54**: 2171-2178.
12. **Gudima, O. S., and V. N. Milyutin.** 1968. Intracellular development of *Rickettsia burnetii* and *prowazekii*, p. 179-184. *In* P. F. Zdrodovsky (ed.), Problems of infectious pathology and immunology. Medgiz, Moscow.
13. **Gulevskaya, S. A., and R. I. Kudelina.** 1967. Submicroscopical structure of *Rickettsia burnetii* I and II phase. J. Microbiol. (Moscow) **12**:133-135.
14. **Handley, J., D. Paretsky, and J. Stueckmann.** 1967. Electron microscopic observations of *Coxiella burnetii* in the guinea pig. J. Bacteriol. **94**:263-267.
15. **Juurlink, B. H. J., and M. A. Hibson.** 1973. Histogenesis of the yolk sac in the chick. Can. J. Zool. **51**:509-519.
16. **Khavkin, T. N., and N. I. Amosenkova.** 1970. Immunofluorescence and phase variation of *Rickettsia burnetii*. Vop. Virusol. **5**:573-578.
17. **Kishimoto, R. A., B. J. Veltri, F. G. Shirey, P. G. Canonico, and J. S. Walker.** 1977. Fate of *Coxiella burnetii* in macrophages from immune guinea pigs. Infect. Immun. **15**:601-607.
18. **Kordova, N.** 1978. Chlamidiae, rickettsiae, and their cell wall defective variants. Can J. Microbiol. **24**:339-352.
19. **Kordova, N., and P. R. Burton.** 1974. Molecular rickettsiology, p. 347-385. *In* J. B. G. Kwapinski (ed.), Molecular microbiology. John Wiley & Sons, Inc., New York.
20. **Lambson, R. O.** 1970. An electron microscopic study of the endodermal cells of the yolk sac of the chick during incubation and after hatching. Am. J. Anat. **129**:1-20.
21. **Leyck, W., and R. Scheffler.** 1975. Multiplication of *Coxiella burnetii* in different tissues and organs of embryonated eggs. Zentralbl. Bakteriol. Hyg. Abt. 1 Orig. Reihe A **231**:519-524.
22. **McDonald, T. L., and L. P. Mallavia.** 1975. Host response to infection by *Coxiella burnetii*. Can. J. Microbiol. **21**:675-681.
23. **Moxon, L. A., A. E. Wild, and B. S. Slade.** 1976. Localization of proteins in coated micropinocytotic vesicles during transport across rabbit yolk sac endoderm. Cell Tissue Res. **171**:175-179.
24. **Nermut, M., S. Schramek, and R. Brezina.** 1972. Further investigations on the fine structure of *Coxiella burnetii*. Zentralbl. Bakteriol. Hyg. Abt. 1 Orig. Reihe A **219**:211-226.
25. **Ormsbee, R. A.** 1952. The growth of *Coxiella burnetii* in embryonated eggs. J. Bacteriol. **63**:73-86.
26. **Romanoff, A. L., and A. J. Romanoff.** 1972. Pathogenesis of the avian embryo; an analysis of causes of malformations and prenatal death. Wiley-Interscience, New York.
27. **Stocker, M. G. P., and P. Fiset.** 1956. Phase variation of the Nine Mile and other strains of *Rickettsia burnetii*. Can. J. Microbiol. **2**:310-321.
28. **Weiss, E.** 1973. Growth and physiology of rickettsiae. Bacteriol. Rev. **37**:259-283.
29. **Weiss, E., and H. Pietryk.** 1957. Growth of *Coxiella burnetii* in monolayer cultures of chick embryo entodermal cells. J. Bacteriol. **72**:235-241.
30. **Wiebe, M. E., P. R. Burton, and D. M. Shankel.** 1972. Isolation and characterization of two cell types of *Coxiella burnetii* phase I. J. Bacteriol. **110**:368-377.
31. **Wild, A. E., and P. Dawson.** 1977. Evidence for Fc receptors on rabbit yolk sac endoderm. Nature (London) **268**:443-445.
32. **Wisseman, C. L., Jr.** 1968. Some biological properties of rickettsiae pathogenic for man. Zentralbl. Bakteriol. Abt. 1 Orig. **206**:299-313.