Acta virol. **28** : 159—173, 1984

RICKETTSIACEAE AND *CHLAMYDIACEAE*: COMPARATIVE ELECTRON MICROSCOPIC STUDIES

A. A. AVAKYAN, V. L. POPOV

N. F. Gamaleya Research Institute of Epidemiology and Microbiology of the U.S.S.R. Academy of Medical Sciences, 123098 Moscow, U.S.S.R.

Received June 1, 1983; revised August 1 , 1983

Summary. — The structure and cytopathology of obligate intracellular bacteria belonging to families *Rickettsiaceae* and *Chlamydiaceae* and their interaction with eukaryotic host cells were compared in electron microscopic studies. "Rickettsia-like" and "chlamydia-like" types of organization of bacterial cells and their interaction with host cells are presented. The rickettsia-like type is characterized by short rod-shaped cells multiplying freely (extravacuolarly) in the cytoplasm or nucleoplasm of the host cell; the chlamydia-like type has spherical cells multiplying inside the cytoplasmic vacuole limited by the host membrane. The rickettsia-like type includes the genus *Rickettsia* and rod-shaped symbionts from genera *Wolbachia* and *Symbiotes*; the chlamydia-like type falls into genera *Chlamydia, Ehrlichia, Cowdria* and *Neorickettsia*. The transitional types represented by *Wolbachia persica* (type 1), *Coxiella* and *Rickettsiella* (type 2) are also described. The possible evolutional relationships of the genera comprising both families are considered and their classification is proposed.

Key words: Rickettsiaceae; Chlamydiaceae; ultrastructure; systematics; interaction with host cell; cytopathology

Rickettsiaceae and *Chlamydiaceae* are two families of bacteria which have adapted to obligatory parasitism in eukaryotic host cells. According to recent electron microscopic observations, many members of the family *Rickettsiaceae* (from tribes *Ehrlichieae* and *Wolbachieae*, Table 1) are more "chlamydia-like" than "rickettsia-like". For differentiation between rickettsiae and chlamydiae, the morphological criteria include signs such as (1) normal anatomy (2) formation and structure of altered (abnormal) forms and (3) features of interaction with host cells. The latter sign comprises the localization of the parasite in the cell (nucleus, free in cytoplasm, in cytoplasmic vacuoles) and the features of the developmental cycle. These criteria become decisive when newly found organisms (as a rule, by electron microscopic examinations of infected animal or plant tissues) are to be classified into one of the two families and orders, respectively.

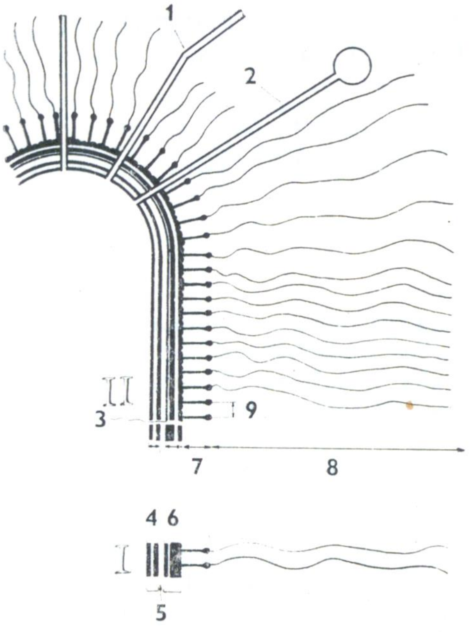

Fig. 1.
Schematic structure of the rickettsial cell
I — *Rickettsia tsutsugamushi* (type A asymetry of the cell wall in ultrathin sections), II — other members of the genus *Rickettsia* (type B asymmetry of cell wall).
1 — fimbria (6—7.5 nm and 8—10 nm in diameter and up to 3 μm in length), 2 — pili (cross size 8—10 nm, diameter of distal sphere 25—30 nm), 3 — rigid layer (peptidoglycan), 4 — cytoplasmic membrane (6—10 nm thick), 5 — periplasmic space (2—4 nm thick), 6 — cell wall (10—13 nm thick), 7 — microcapsule [10—30 nm thick with distance of 13 nm between subunits (9)], 8 — slime layer (150—300 nm thick).

This review deals with differentiation and determination of the criteria of "rickettsia-likeness" and "chlamydia-likeness" possible by comparing the ultrastructural organization of these organisms according to the above signs.

Structure of normal forms

Rickettsia are short rods, usually 0.3—0.5 × 0.8—1.5 μm in size, sometimes with thickening in the middle part of the cell. Due to their structure, rickettsial cells are similar to Gram-negative bacteria (Fig. 1). Recently a considerable variability of their surface structures has been found. All rickettsiae appear to have fimbria (Gudima and Milyutin, 1968; Kokorin and Gudima, 1968; Gudima, 1969; Gudima and Pereverzev, 1976). *R. prowazekii* display pili morphologically similar to sex pili of *Escherichia* (Gudima and Pereverzev, 1976). Rickettsiae, like most of bacteria (Costerton *et al.*, 1981) have a thick slime layer or a capsule that was demonstrated after treatment with specific antibodies (Silverman *et al.*, 1978; Rikihisa *et al.*, 1979). Under it, directly on the cell wall surface, the microcapsule is situated showing a certain periodicity in structure detectable in ultrathin tangential sections (Gudima *et al.*, 1973); this can be seen especially clearly in negatively stained preparations of plasmolysed rickettsiae (Palmer *et al.*, 1974) and in ruthenium red-stained cells. Based on the cytochemical reaction of the capsule and microcapsule (Popov and Ignatovich, 1976; Silverman *et al.*, 1978; Čiampor *et al.*, 1978), they contain acid polysaccharides.

The cell wall surface layer sometimes has a globular structure which is

Table 1. The system of rickettsia and chlamydia (modified according to Bergey, 1974)

Order	Family	Tribe	Genus	Species
Rickettsiales	Rickettsiaceae	Rickettsiae	Rickettsia	prowazeki typhi canada rickettsii sibirica conorii parkeri australis akari montana rhipicephali slovaka tsutsugamushi
			Rochalimaea	quintana vinsonii
			Coxiella	burnetii
		Ehrlichiae	Ehrlichia	canis phagocytophila sennetsu
			Cowdria	ruminantium
			Neorickettsia	helminthoeca
		Wolbachiae	Wolbachia	pipientis melophagi persica
			Symbiotes	lectularius
			Blattabacterium	cuenoti
			Rickettsiella	popilliae
			Porochlamydia	buthi
Chlamydiales	Chlamydiaceae		Chlamydia	psittaci trachomatis

To the list of species given in Bergey Manual, the newly isolated species and genus have been added: *Rickettsia montana*, *R. rhipicephali*, *R. slovaka*, *Rochalimaea vinsonii* (previously known as a vole agent), and *Porochlamydia buthi*. It was suggested that *Rickettsia sennetsu* should be classified into the genus *Ehrlichia* on the basis of morphological and antigenic similarity (Hoilien *et al.*, 1982).

also typical of most bacteria (Sleytr, 1978). A diameter of the globuli is 4.5 nm in *R. prowazekii* and *R. typhi* and 3 nm in *R. tsutsugamushi* (Gudima, 1969; Gudima and Pereverzev, 1976). In ultrathin sections the cell wall has an asymmetric profile: in typhus and spotted fever groups of rickettsiae thicker is the internal osmiophilic layer of the membrane (type B asymmetry)

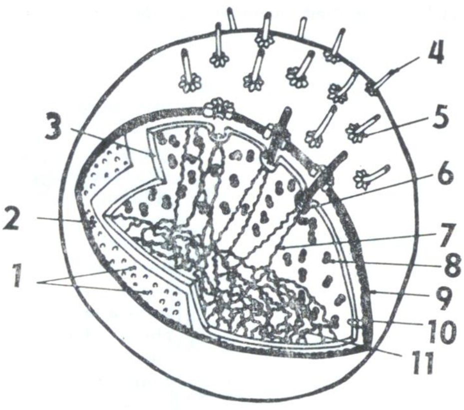

Fig. 2.

Schematic organization of chlamydial elementary body (from Matsumoto 1982*b*, with the author's permission) 1 — particles of the intermediate layer, 10 nm in diameter, 2 — intermediate layer, 3 — cytoplasmic membrane (8 nm thick), 4 — tubular projection (35 to 40 nm in length, cross size 5—6 nm, diameter of the inner canal 2.5—3.5 nm, the wall is formed by 8—9 helically arrayed subunits), 5 — "flower" or "rosette" (10—12 nm in external diameter, 6.5 nm internal diameter), 6 — site of attachment of the projection to the cytoplasmic membrane (B-structure), 7 — DNA strand attached to the B-structure, 8 — ribosome, 9 — cell wall (in ultrathin sections 8 nm thick), 10 — a layer of flat rounded subunits of the cell wall membrane having a diameter of 20 nm and packed hexagonally, 11 — nucleoid.

(Gudima and Alimov, 1974), while in *R. tsutsugamushi* the external one (type A asymmetry) (Brinton and Burgdorfer, 1971; Avakyan *et al.*, 1973*a*; Gudima *et al.*, 1974; Silverman and Wisseman, 1978; Ito *et al.*, 1978; Rikihisa *et al.*, 1979; Hayes and Burgdorfer, 1979). Type B asymmetry is readily explainable by the fact that osmiophilia of the internal layer of the cell wall membrane under conventional fixation conditions frequently masks the peptidoglycan layer. In *R. tsutsugamushi* peptidoglycan is not detectable in ultrathin sections and may possibly have a peculiar structure. This is supported by the insusceptibility of *R. tsutsugamushi* to high doses of penicillin 500—1 000 μg/ml) as documented, in addition to other methods, also by electron microscopic studies (Silverman and Wisseman, 1978).

Chlamydia exist in two vital forms regularly alternating in the developmental cycle: vegetative (in the form of reticulate bodies, RB) and spore-like (in the form of elementary bodies, EB). RB have two main biological features: the lack of infectivity and marked metabolic activity. In contrast, EB are infectious but have limited metabolic potentials (Becker, 1978). These two forms differ ultrastructurally but at the same time are similar in both chlamydia species. EB are small rigid spherical cells 0.25—0.4 μm in diameter with a dense excentrical nucleoid. In ultrathin sections of inclusions of some strains more minute dense EB of irregular shape are found. As a rule, these EB have dense limiting membranes. Sometimes EB with stellate outlines are observed (Eb *et al.*, 1976; Popov, 1979). Usually we found such EB forms in newly isolated strains when first adapted to cell cultures or yolk sac epithelium. RB are plastic spherical cells 0.6—1.5 μm in diameter ultrastructurally similar to Gram-negative bacteria. As shown by cryoultramicrotomy, RB may have most variably shapes depending on the density of packing in inclusions (Popov *et al.*, 1978).

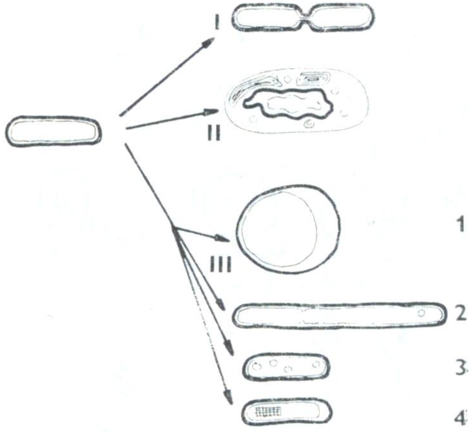

Fig. 3.
Three patterns of rickettsiae-host cell interaction
I — reproduction, II — destruction of rickettsiae (in phagolysosomes), III — generation of altered (abnormal) forms: 1 — spheroplast-like, 2 — filamentous, 3 — vacuolated, 4 — containing crystalloid structures in the cytoplasm. Patterns I, II, and III may be observed simultaneously in the same host cell.

Chlamydiae were found to have no pili, fimbriae or flagellae but possess peculiar tubular projections penetrating openings in the cell wall ("rosettes") and apparently attaching to the cytoplasmic membrane (Fig. 2). The tubular projections are found both in EB and RB, on the surface of the former they are arranged in 1—2 groups of about 18 projections in each. On RB they were frequently randomly arranged, particularly in the early stages of the developmental cycle, and their number at this time (10 hr post-infection) reached 45 and then gradually decreased (Matsumoto, 1979; 1982*a*, *b*). Their function is obscure. Preliminary studies (Gregory *et al.* 1979) showed them to play no role in the attachment of EB to the host cell. Tubular projections of RB may penetrate the inclusion membrane and thus apparently serve as channels for transfer of metabolites from the host cytoplasm (Matsumoto, 1981). A layer of polysaccharides corresponding to the microcapsule (Popov, 1979) and containing the genus-specific antigen which can be detected by the immunoferritin (Higashi *et al.*, 1966) and immunoperoxidase (Shatkin *et al.*, 1976*a*; Popov *et al.*, 1976; Richmond and Stirling, 1981) procedures is found on EB and RB surface.

The EB cell wall is externally formed by particles 5—6 nm in diameter. The internal surface of its membrane has a layer of rounded subunits 20 nm in diameter packed hexagonally. It is apparently this layer that provides the rigidity of EB cell wall because penicillin prevents its formation when RB become EB (Matsumoto, 1982*b*). Sometimes, ultrathin sections of EB showed a very dense intermediate layer of permanent thickness between cell wall membrane and cytoplasmic membrane as if combining both membranes into a single envelope complex (Poffenroth *et al.*, 1973; Shatkin *et al.*, 1976*b*; Popov, 1979). This structure was similar to an analogous complex in coxiellae and rickettsiellae (Avakyan *et al.*, 1983).

A cytoplasmic membrane surrounds the sites of attachment of the tubular projections ("B-structures") and has here peculiar properties, for in-

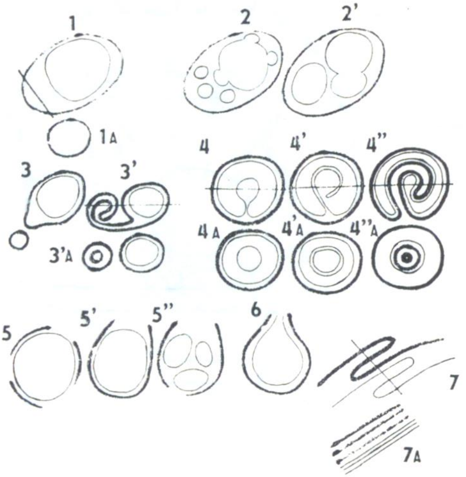

Fig. 4.
Mechanisms of generation of abnormal forms of chlamydiae
Expansion of the periplasmic space and its cross sections (1, 1A). Budding from the protoplast of small or larger forms into the periplasmic space (2, 2'). Formation of evaginations of various length by the cell wall membrane (3, 3') cross-sections of which may look like vesicles limited by one or two membranes (3, 3'A). Formation of various invaginations of the cytoplasmic membrane into the protoplast (4, 4') or deep invagination of the cell envelope (cell wall-cytoplasmic membrane) (4''). In cross-sections such forms will appear as a protoplast fragmented by one or several membranes (4A, 4'A, 4''A). Formation of spheroplast-like or protoplast-like structures upon destruction of the cell wall (5, 5', 5'') or destruction of the entire large form. Breaks of cell wall and cytoplasmic membrane (6). Parallel formation of evaginations by cell wall membrane and cytoplasmic membrane (7) which in cross-sections appear as multilayered membranes limiting some "giant" forms (7A).

stance, resistance to detergents which destroy it in the other parts. In these sites, the chromosome is attached to its internal surface by means of a trypsin-sensitive component (Matsumoto, 1982*b*).

Typical EB were found only in chlamydiae, however, for coxiellae and rickettsiellae, apart from conventional cells ("clear large"), rod-shaped dense small cells have been described (Wiebe *et al.*, 1972; Devauchelle *et al.*, 1972; McCaul and Williams, 1981). Although dense cells of coxiellae are infective, they differ from clear cells by a higher content of peptidoglycan, lower metabolic activity and lower sensitivity to osmotic shock (McCaul and Williams, 1981).

All the members of *Ehrlichieae* tribe have round cells 0.5 to 1 μm in diameter and ultrastructurally similar to chlamydial RB. Rickettsia-like symbionts of the genus *Wolbachia* may be coccoid and rod-shaped, most cells with typical electron dense parts of nucleoid (Avakyan *et al.*, 1973*b*). In *Cimex lectularius* mycetomas 2 types of symbionts were found (Chang and Musgrave, 1973): rod-shaped rickettsia-like which may be considered to be *Symbiotes lectularius*, and rounded chlamydia-like ones.

Formation and structure of abnormal forms

When the optimal conditions are changed, and in particular under non-permissive conditions, altered (abnormal) forms may appear. Four types of altered forms may be found among rickettsiae (Fig. 3-II): filamentous, vacuolated, containing crystalline structures, and spheroplast-like. Filamentous rickettsiae seem to be formed as a result of disturbed mechanism of cell fission. For example, when fresh medium is added, the cytokinesis is restored and rickettsial cells of normal size appear (Gulevskaya *et al.*, 1975; Kokorin and Kiet, 1976; Silverman *et al.*, 1980). Vacuolated rickettsiae frequently appear at later stages of intracellular development. In studies with *R. typhi* by the freeze-fracture method, vacuoles or small vesicles 40—150 nm in diameter appeared as smooth rounded indentations without signs of a limiting membrane (Ito *et al.*, 1975; 1978). Crystalloid structures of different degrees of complexity have been described in the cytoplasm of many rickettsial species. At least 3 types of organization of these structures are distinguished: amorphous granular, linear, and "microtubular-like" (Gulevskaya and Ignatovich, 1971; Brinton and Burgdorfer, 1971; Avakyan *et al.*, 1973*a*). Different types of organization may possibly reflect different stages of their genesis and packing. They were usually found in cells with altered morphology. The origin of vacuoles and crystalls has not been determined definitely, although it was assumed that vacuoles might play the role of storage granules (Silverman *et al.*, 1974; Ito *et al.*, 1975) and crystalls are formed by protein (Gulevskaya and Ignatovich, 1971; Brinton and Burgdorfer, 1971; Louis *et al.*, 1977). Spheroplast-like forms have regularly been found in rickettsia-infected cells in their normal interaction (Gulevskaya *et al.*, 1975; Silverman *et al.*, 1980; Popov and Barkhatova, 1981) and in *Rochalimea* colonies (Ito and Vinson, 1965). The regular finding of spheroplasts which may be considered to be rickettsial forms of unbalanced growth could also represent the initial stage of their L-transformation (Prozorovsky *et al.*, 1981) suggesting, as in bacteria, the existence of L forms of rickettsiae.

The altered forms of chlamydiae are represented by giant spheroplast-like bodies (up to 5 μm in diameter), "minute" forms (100—250 nm) frequently located in expansions of the periplasmatic space of spheroplast-like forms, and vesicular structures (30—80 nm) in the inclusion cavity (Popov *et al.*, 1977). Analysis of the formation mechanism of these structures (Fig. 4) (Beskina *et al.*, 1979) and their morphological similarity to the forms observed in bacterial L-transformation allow them to be considered as manifestation of L-transformation of chlamydiae (Prozorovsky *et al.*, 1979). The altered forms of this type have been reported in *Ehrlichia equi* (Sells *et al.*, 1976), the giant (pleomorphic) forms in *Cowdria* (Pienaar, 1970), *Wolbachia* sp. (Hayes and Burgdorfer, 1979) and *Rickettsiella* (Devauchelle *et al.*, 1972).

From these observations it is difficult to agree with the assumption of Kordová (1978) that vegetative forms of rickettsiae and chlamydiae are the L-forms themselves. Most likely, in the course of adaptation to intracellular parasitism they had undergone changes in the composition and structure of the cell walls, which is particularly true for chlamydiae. As a result of these changes the processes of cell wall growth and cell fission are readily disturbed. Owing to this, upon endo- and exogenic effects on host cell, to which facultative intracellular bacteria do not react, rickettsiae and chlamydiae produce spheroplast-like (giant, pleomorfic) forms.

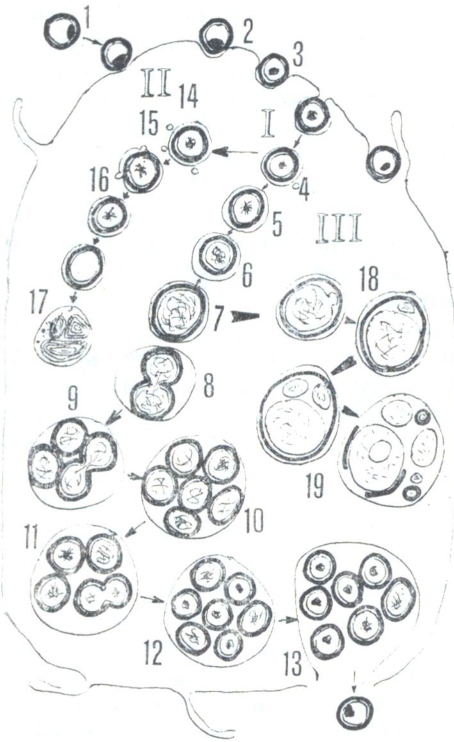

Fig. 5.
Three kinds of interaction of chlamydiae with the host cell
I — reproduction (developmental cycle) (1—13), II — destruction in phagolysosomes (14—17), III — L-transformation (18—19).
1 — absorption on plasmalemma of EB with compact nucleoid and rigid cell wall with a diameter of 0.25—0.4 μm; 2, 3 — endocytosis; 4 — EB in the phagocytic vacuole (phagosome); 5, 6, 7 — reorganization of EB via a transitional form into RB which has a loose nucleoid plastic cell wall and a size of 0.4—0.8 μm (beginning of the vegetative stage of the cycle); 8 — RB binary fission; 9 — formation of the cytoplasmic inclusion-chlamydial microcolony, several cycles of RB fission; 10 — continuation of RB fission, formation of intermediate bodies (IB); 11 — continuation of IB formation, their fission, formation of EB; 12 — EB "maturation"; 13 — release of a new EB generation into the extracellular space through the break of the inclusion membrane and plasmalemma of the host cell; 14 — approach of primary lysosomes to the phagocytic vacuole (phagosome); 15 — fusion of primary lysosomes with phagosome and formation of phagolysosome; 16 — destruction of chlamydiae in phagolysosome; 17 — formation of telolysosome; 18 — increase of RB in size, formation of cell wall evaginations and vesicle-like structures; 19 — formation of polymorphous "giant" forms with detachment of the cell wall, expansions of the periplasmic space, small forms.

Features of interaction with host cells

Rickettsiae and chlamydiae, upon entering the cell, are initially present inside phagosomes. Their further fate may be as follows: I — reproduction (for which rickettsiae first have to leave the phagosome), II — destruction in phagolysosomes, and III — generation of abnormal forms (Figs. 3, 5, 6) (Popov *et al.*, 1980; Popov and Barkhatova, 1981). Theoretically, a fourth way is also possible: survival without multiplication. The latter is important for persistence of intracellular parasites, however no definite ultrastructural data confirming it are available.

Rickettsiae multiply freely in the cytoplasm of the nucleus (extravacuolarly) of the host cell by binary transversal fission. There is no obligate cycle of development. A facultative "infection cycle" may be observed which consists in alternation of vegetative reproducing cells (the exponential phase forms) and dormant forms (cells of the stationary phase of growth) (Gudima and Milyutin, 1968; Wisseman and Waddell, 1975). *Coxiella*, *Ehrlichia*, *Cowdria*, *Neorickettsia*, *Wolbachia*, *Symbiotes*, *Rickettsiella* and *Chlamydia* replicate only within the cytoplasmic vacuole limited by the host membrane. Chlamydiae are characterized by the developmental cycle consisting of mandatory alternation of vegetative and spore-like forms (Fig. 5-I) (Friis, 1972; Storz and Snears, 1977; Popov *et al.*, 1980). In coxiellae, despite the existence of two types of cells equally capable of fission, no regular alternations of them were observed, but recently a peculiar cycle of "sporogenic" differentiation has been described in which dense minute "endospores" (130—170 nm) were formed in the periplasmic space of some large cells

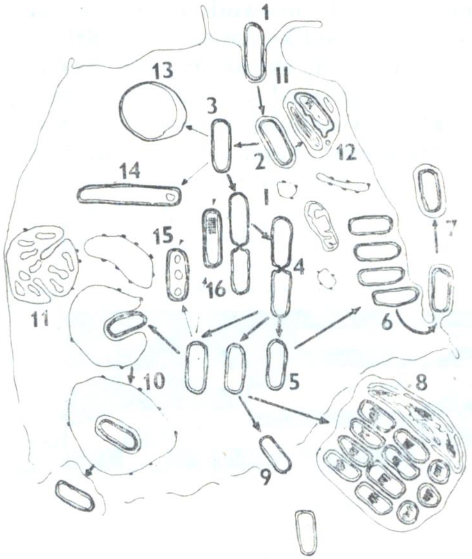

Fig. 6.

Rickettsiae — host-cell interaction

I — Reproduction (1—10), II — destruction in the phagolysosome system of the host cell (12); III — generation of abnormal forms (13—16).
1 — absorption of rickettsia on the host cell surface and penetration by phagocytosis; 2 — rickettsia inside phagosome in the host cell cytoplasm; 3 — release of rickettsia from the phagosome; 4, 5 — binary fission of rickettsiae (several cell cycles) lying free in the cytoplasm and formation of a new generation of rickettsiae; 6, 7, 8, 9, 10 — rickettsial release from the cell: 6 — release of mobile rickettsiae by "budding" from plasmalemma surface, rickettsiae in the extracellular space are surrounded by the host plasmalemma (7); 8 — formation of "Mooser's cells" the cytoplasm of which is densely packed with rickettsiae usually in dormant forms; 9 — release of rickettsiae through plasmalemma breaks; 10 — release of *R.rickettsii* into the cisterns of granular endoplasmic reticulum, rickettsiae being surrounded by internal membranes of the host cell, and large vacuoles are formed in the host cell cytoplasm (11); 12 — rickettsial destruction in phagolysosome and formation of telolysosome; 13 — generation of spheroplast-like forms of rickettsiae; 14 — generation of filamentous forms of rickettsiae; 15 — formation of vacuolated rickettsiae; 16 — formation of crystalloid structures in the cytoplasm of rickettsiae.

(McCaul and Williams, 1981). Rickettsiellae have a mandatory developmental cycle consisting of a regular alternation of small rod-shaped dense forms and bacteria-like ones which undergo fission and then again reorganize into dense forms (Devauchelle *et al.*, 1972).

Classification comments

Analysis of the results of electron microscopic examinations of all members of the *Rickettsiaceae* and *Chlamydiaceae* families according to the foregoing morphological criteria shows the most genera presently included into the family *Rickettsiaceae* to be more similar to *Chlamydia* than to *Rickettsia*. This similarity can be followed in several features (Fig. 7) despite the fact that the majority of these genera (with the exception of *Rickettsiella* and *Coxiella*) have no spore-like forms and, consequently, no mandatory alternation of vegetative and spore-like forms, i.e. no developmental cycle. Summing up the results of studies at the submicroscopic level, we can distinguish and characterize the "rickettsia-like" and "chlamydia-like" types of cell organization of obligate intracellular bacteria and their interaction with the host cell. The "rickettsia-like" type are short rod-shaped cells multiplying

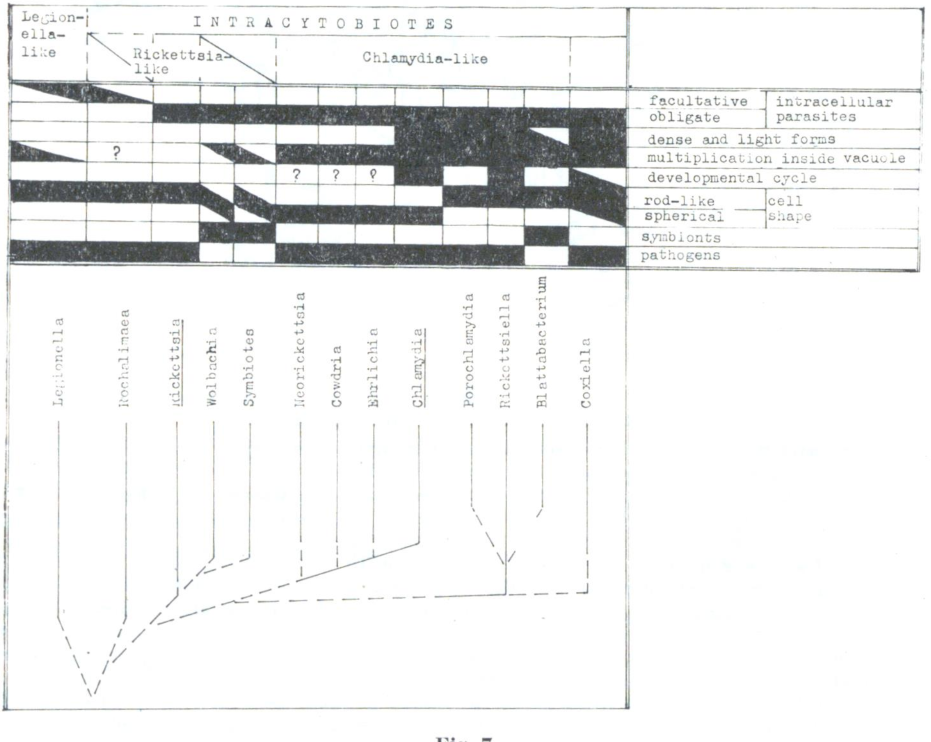

Fig. 7.
A scheme of possible relationships between genera in the class *Intracytobiotes*

freely (extravacuolarly) in the cytoplasm or nucleoplasm of the host cell. The "chlamydia-like" type is represented by spherical cells multiplying inside the cytoplasmic vacuole limited by the host cell membrane.

The rickettsia-like type includes the genus *Rickettsia* and rod-shaped symbionts from the genera *Wolbachia* and *Symbiotes*, the chlamydia-like type the genera *Chlamydia*, *Ehrlichia*, *Cowdria* and *Neorickettsia*. At the same time, transitional types may be distinguished: (1) *Wolbachia persica* may have both-rod-shaped and spheroid forms and develop both intra- and extravacuolarly. They multiply inside the vacuole but it is not known whether the initiation of multiplication requires any direct contact of wolbachiae with the host cell cytoplasm, i.e. release from the vacuole; (2) *Coxiella* and *Rickettsiella* have 2 types of cells, both rod-shaped, and are quite similar to each other in the organization of small dense cells and large clear cells as well as in multiplication inside the vacuole, but coxiellae have no obligate developmental cycle. The dense cell envelope typical of small cells of these organisms is also found under certain conditions in chlamydiae which may possibly attest to relationships of these three genera. Noteworthy is the fact that both *Coxiella* and *Chlamydia* have similar content of G + C in their DNA; for *Rickettsiella* this has not been determined yet.

If the developmental cycle of chlamydia, which currently some research workers regard for unique among bacteria, was compared with the infectious cycle of *R. prowazekii*, some principal similarity could be found probably determined by changes in the structure of bacteria in the exponential and stationary phases of the culture growth. These changes had been studied at length in early works by light microscopy but not at the submicroscopic level. It was shown by light microscopy (Jawetz *et al.*, 1980) that in the stationary phase of growth bacteria frequently became smaller, denser, acquiring a rounded shape and intracytoplasmic granules, and became more resistant to physical effects and chemical agents. In rickettsiae, probably same as in saprophyte bacteria, this process occurs under unfavourable conditions, for instance, upon exhaustion of nutrient substances, whereas in chlamydia, possibly owing to their greater metabolic lability, this feature had stabilized, and the mandatory developmental cycle emerged. The similarity of the developmental cycles of rickettsiellae and chlamydiae is hardly convergent but rather attests to relationship of these genera.

Sometimes chlamydial inclusions contain smaller dense EB of irregular shape surrounded by a dense "envelope". Such cells may be more virulent. This is probably true of rickettsiae as well: purified smaller and denser cells of *R. prowazekii* with sinuous outlines have been shown to be more virulent (Hanson *et al.*, 1981).

The performed differentiation of rickettsia-like and chlamydia-like organisms with due consideration of the data on morphology of extra- and intracellular developmental forms permits an attempt at their classification. It should be emphasized that this attempt refers only to the above-described criteria and is not based either on biochemical and biophysical analysis of the structures or on the analysis of their antigenic properties. Neverheless, it seems possible even now to classify into the family *Chlamydiaceae* (and, consequently, to the order *Chlamydiales*) the entire *Ehrlichieae* tribe as well as the genera *Rickettsiella* and *Coxiella* separating them into a special group, e.g., a tribe. Then the family *Chlamydiaceae* will consist of three tribes including the following genera: *Chlamydiae* trib. nov. (*Chlamydia*), *Ehrlichiae* (*Ehrlichia, Cowdria, Neorickettsia*), *Rickettsielleae* trib. nov. (*Rickettsiella, Porochlamydia, Coxiella*).

Possible associations of the genera comprising the families *Rickettsiaceae* and *Chlamydiaceae* and some of their characteristics are shown in Fig. 7. A special position in this group is occupied by *Rochalimaea* which is a facultative intrecellular parasite and therefore may be more associated with the ancestral form of rickettsiae which is probably related to the modern *Legionella* and should be classified together with them. *Rochalimaea* can hardly be united in one taxon of the order rank with *Rickettsiaceae*, because their associations are at present obscure. This problem requires further study.

We believe to be absolutely right from the point of view of evolution and advisable for classification to unite *Rickettsiaceae* and *Chlamydiaceae* into a special class of obligate intracellular scotobacteria (Bergey, 1974) which we suggest to be named *Intracytobiotes* nom. nov. (Shatkin and Popov, 1982). On the other hand, the formulated criteria of "rickettsia-likeness" and "chlamydia-likeness" should facilitate differentiation of rickettsia, chlamydia, rickettsia-like and chlamydia-like symbionts and pathogens in invertebrates, especially vectors as well as other organisms in wildlife.

Acknowledgement. The authors are cordially grateful to Professors I. N. Kokorin and A. A. Shatkin, Doctors N. M. Balaeva, V. F. Ignatovich, and L. N. Kats for the critical discussion of the manuscript.

References

Avakyan, A. A., Gudima, O. S., and Alimov, Zh. A. (1973*a*): Electron microscopic study of *Rickettsia canada* in tissue culture cells (in Russian). *Zh. Microbiol (Mosk.)* **1973** (3), 3—7.

Avakyan, A. A., Sidorov, V. E., and Chebanov, S. M. (1973*b*): Regularities of intracellular symbiosis of rickettsia-like symbionts and *Argasid* mites (in Russian). *Dokl. Akad. Nauk SSSR*, **211** (3), 707—710.

Avakyan, A. A., Popov, V. L., Chebanov, S. M., Shatkin, A. A., Sidorov, V. E., and Kudelina, R. I. (1983): Comparison of ultrastructure of obligate intracellular prokaryotes, *Chlamydia* and *Coxiella*. *Acta virol.* **27**, 168—172.

Becker, Y. (1978): The Chlamydia: molecular biology of prokaryotic obligate intracellular parasites of eukaryocytes. *Microbiol. Rev.* **42**, 274—306.

Bergey's Manual of Determinative Bacteriology, pp. 882—918, Buchanan & Gibbons (Eds), Williams & Wilkins Co., Baltimore, 8th ed., 1974.

Beskina, S. R., Barkhatova, O. I., Popov, V. L., Shatkin, A. A., and Prozorovsky, S. V. (1979): The effect of penicillin on the paratrachoma agent in the time course of infection in vitro (in Russian). *Antibiotiki* **24** (8), 598—603.

Brinton, L. P., end Burgdorfer, W. (1971): Fine structure of *R. canada* in tissues of *Dermacentor andersoni* Stiles. *J. Bact.* **105**, 1149—1159.

Chang, K. P., and Musgrave, A. J. (1973): Morphology, histochemistry, and ultrastructure of mycetome and its rickettsial symbiotes in *Cimex lectularius* L. *Can. J. Microbiol.* **19**, 1075 to 1081.

Čiampor, F., Schramek, Š., and Brezina, R. (1978): Electron microscopy of the ruthenium red-stained cell wall of typhus group rickettsiae, pp. 49—52. In Kazár, R. A. Ormsbee (Eds): *Rickettsiae and Rickettsial Diseases*, VEDA, Bratislava.

Costerson, J. W., Irwin, R. T., and Cheng, K. J. (1981): The role of bacterial surface structures in pathogenesis. *CRC Crit. Rev. Microbiol.* **8**, 303—338.

Devauchelle, G., Meynadier, G., and Vago, C. (1972): Etude ultrastructurale du cycle de multiplication de *Rickettsiella melolonthae* dans les hémocytes de son hôte. *J. uetrastr. Res.* **38**, 134—148.

Eb, F., Orfila, J., and Lefebvre, J. F. (1976): Ultrastructural study of the development of the agent of ewe's abortion. *J. Ultrastr. Res.* **56**, 177—185.

Friis, R. R. (1972): Interaction of L-cells and *Chlamydia psittaci*: entry of the parasite and host responses to its development. *J. Bact.* **110**, 706—721.

Gregory, W. W., Gardner, M., Byrne, G. J., and Moulder, J. W. (1979): Arrays of hemispheric surface projections on *Chlamydia psittaci* and *C. trachomatis* observed by scanning electron microscopy. *J. Bact.* **138**, 241—244.

Gudima, O. S. (1969): Features of the structural organization of *Rickettsiae* (in Russian). *Vest. Akad. med. Nauk SSSR*, **10**, 35—40.

Gudima, O. S., Alimov, Zh. A., and Avakyan, A. A. (1973): Ultrastructure of *Rickettsia mooseri* (in Russian). *Zh. Mikrobiol.* (*Mosk.*) **1973**, (10), 88—91.

Gudima, O. S., and Alimov, Zh. A. (1974): Ultrastructure of the agent of vesicular rickettsiosis, *Dermacentroxenus murinus* Kulagin, 1951, and some features of its interaction with the cell (in Russian). *Zh. Mikrobiol.* (*Mosk.*) **1974** (7), 8—10.

Gudima, O. S., Alimov, Zh. A., and Avakyan, A. A. (1974): Structural organization of *Rickettsia tsutsugamushi* and features of its interaction with the cell (in Russian). *Zh. Mikrobiol.* (*Mosk.*) **1974** (8), 9—11.

Gudima, O. S., and Milyutin, V. N. (1968): Intracellular development of *Rickettsia burneti* and *Rickettsia prowazeki* (in Russian). In: *Vop. infekt. Pat. Immunol.* **4**, 290—298.

Gudima, O. S., and Pereverzev, N. A. (1976): Surface structures of typhus and tsutsugamashi group rickettsiae (in Russian-. In: *Nop. infekt. Pat. Immunol.* **5**, 290—298.

Gulevskaya, S. A., and Ignatovich, V. F. (1971): New electron microscopic data on structural formations in the cytoplasm of *Rickettsia prowazeki*. *Acta virol.* **15**, 510—514.

Gulevskaya, S. A., Popov, V. L., and Ignatovich, V. F. (1975): New data on polymorphism of *Rickettsia prowazeki* and *R. burneti* propagated in cell culture (in Russian). *Zh. Microbiol.* (*Mosk.*) **1975** (7), 68—72.

Hanson, B. A., Wisseman, C. L., and Waddell, A., and Silverman, D. J. (1981): Some characteristics of heavy and light bands of *Rickettsia prowazekii* on renografin gradients. *Infect. Immun.* **34**, 596—604.

Hayes, S. F., and Burgdorfer, W. (1979): Ultrastructure of *Rickettsia rhipicephali*, a new member of the spotted fever group rickettsiae in tissues of the host vector *Rhipicephalus sanguineus*. *J. Bact.* **137**, 605—613.

Higashi, N., Masumoto, A., Nagatomo, Y., and Fujiwara, E. (1966): Immune electron microscopic studies on the PLT group agent using ferritin-conjugated antibody technique. *Ann. Rep. Inst. Virus. Res., Kyoto Univ.* **9**, 154—156.

Hoilien, C. A., Ristic, M., Huxsol, D. L., and Rapmund, G. (1982): *Rickettsia sennetsu* in human blood monocyte culture: similarities to the growth cycle of *Ehrlichia canis*. *Infect. Immun.* **35**, 314—319.

Ito, S., and Vinson, J. W. (1965): Fine structure of *Rickettsia quintana* cultivated in vitro and in the louse. *J. Bact.* **89**, 481—495.

Ito, S., Vinson, J. W., and McGuire, T. J., Jr. (1975): Murine typhus in the oriental rat flea. Pathobiology of invertebrate vectors of diseases. *Ann. N.Y. Acad. Sci.* **266**, 35—60.

Ito, S., Vinson, J. W., and Whitescarver, J. (1978): Ultrastructural observations of *Rickettsia typhi* in the human body louse, pp. 53—64. In J. Kazár, R. A. Ormsbee, I. V. Tarasevich (Eds): *Rickettsiae and Rickettsial Diseases*, VEDA, Bratislava.

Jawetz, E., Melnick, J. L., and Adelberg, E. A. (1980): Review of medical microbiology, p. 59. Lange Medical Publ.

Kokorin, I. N., and Gudima, O. S. (1968): Electron microscopic examination of *D. murinus* stained with phosphotungstic acid (in Russian). *Zh. Mikrobiol.* (*Mosk.*) **1968** (12), 5—8.

Kokorin, I. N., and Chyong dinh Kyet (1976): Vital observations and time-lapse cinemicrography of intracellular development of *D. murinus* and its interaction with cells (in Russian). *Zh. Mikrobiol.* (*Mosk.*) **1976** (5), 50—52.

Kordová, N. (1978): Chlamydiae, rickettsiae, and their cell wall defective variants. *Can. J. Microbiol.* **24**, 339—352.

Louis, C., Croizier, G., and Meynadier, G. (1977): Trame cristalline des inclusions protéiques chez une *Rickettsiella*. *Biol. Cellulaire* **29**, 77—80.

Matsumoto, A. (1979): Recent progress of electron microscopy in microbiology and its development in future — from a study of the obligate intracellular parasites, *Chlamydia* organisms. *J. Electron Micr.* **28** (suppl.), 557—564.

Matsumoto, A. (1981): Isolation and electron microscopic observations of intracytoplasmic inclusions containing *Chlamydia psittaci*. *J. Bact.* **145**, 605—612.

Matsumoto, A. (1982*a*): Electron microscopic observations of surface projections on *Chlamydia psittaci* reticulate bodies. *J. Bact.* **150**, 358—364.

Matsumoto, A. (1982*b*): Morphology of *Chlamydia psittaci* elementary bodies as revealed by electron microscopy. *Kawasaki Med. J.* **8**, 149—157.

McCaul, T. F., and Williams, J. C. (1981): Developmental cycle of *Coxiella burnetii*: structure and morphogenesis of vegetative and sporogenic differentiations. *J. Bact.* **147**, 1063—1076.

Palmer, E. L., Mallavia, L. P., Tzianabos, T., and Obijeski, J. F. (1974): Electron microscopy of the cell wall of *Rickettsia prowazeki*. *J. Bact.* **118**, 1158—1166.

Pienaar, J. G. (1970): Electron microscopy of *Cowdria* (Rickettsia) *ruminantum* (Cowdry, 1926) in the endothelial cells of the vertebrate host. *Onderstepoort J. vet. Res.* **37**, 67—78.

Poffenroth, L., Costerton, J. W., Kordová, N., and Wilt, J. C. (1973): Ultrastructural studies of *Chlamydia psittaci* 6BC variant strains. I. Ultrastructure of the surface layers of egg-passaged 6BC strain. *Can. J. Microbiol.* **19**, 887—896.

Popov, V., Eb, F., Lefebvre, J. F., Orfila, J., and Viron, A. (1978): Morphological and cytochemical study of Chlamydia with EDTA regressive technique and Gautier staining in ultrathin frozen sections of infected cell cultures. *Ann. Inst. Pasteur* **129**B, 313—337.

Popov, V. L., and Ignatovich, V. F. (1976): Electron microscopy of surface structures of *Rickettsia prowazeki* stained with ruthenium red. *Acta virol.* **20**, 424—428.

Popov, V. L. (1979): Ultrastructure of *Halprowiae* (Chlamydiae) (in Russian), pp. 12—17. In O. V. Baroyan (Ed.): *Halprowioses (Chlamydioses) cheloveka i zhivotnykh*; collected papers, issue 1, Moscow.

Popov, V. L., Beskina, S. R., and Shatkin, A. A. (1976): Electron microscopic determination of localization of the group-specific antigen of *Halprowiae* (*Chlamydiae*) by the immunoperoxidase method (in Russian). *Zh. Mikrobiol.* (Mosk.) **1976** (12), 70—73.

Popov, V. L., Shatkin, A. A., Avakyan, A. A., and Prozorovsky, S. V. (1977): Unusual small forms in the developmental cycle of *Halprowiae* (Chlamydiae) and their possible association with L-transformation phenomena (in Russian). *Zh. Mikrobiol.* (*Mosk.*) **1977** (3), 42—46.

Popov, V. L., Beskina, S. R., Shatkin, A. A., and Avakyan, A. A. (1980): Ultrastructure of the early stages of *Halprowiae* (Chlamydiae) interaction with host cell (in Russian). *Zh. Mikrobiol.* (*Mosk.*) **1980** (8), 28—33.

Popov, V. L., and Barkhatova, O. I. (1981): Interaction of *Rickettsia akari* with host cell in vitro: electron microscopic study, pp. 48—49 (in Russian). In: S. V. Prozorovsky (ed.): *Voprosy rickettsiologii:* collected papers, issue 2.

Prozorovsky, S. V., Beskina, S. R., Popov, V. L., and Barkhatova, O. I. (1979): Morphological changes in *Halprowiae* (Chlamydiae) of the type of bacterial L-transformation (in Russian) pp. 22—26. In: O. V. Baroyan (Ed.): *Halprowioses* (*Chlamydioses*) *cheloveka i zhivotnykh*, issue 1.

Prozorovsky, S. V., Kats, L. N., and Kagan, G. Ya. (1981): L-forms of bacteria: mechanism of formation, structure, role in pathology (in Russian), 240 p. Meditsina Publisher, Moscow.

Richmond, S. J., and Stirling, P. (1981): Localization of chlamydial group antigen in McCoy cell monolayers infected with *Chlamydia trachomatis* or *Chlamydia psittaci*. *Infect. Immun.* **34**, 561—570.

Rikihisa, Y., Rota, T., Lee, T. H., and McDonald, A. B., and Ito, S. (1979): Changes in immunoferritin labeling of *Rickettsia tsutsugamushi* after serial cultivation in ^{60}Co-irradiated BHK cells. *Infect. Immun.* **26**, 638—650.

Sells, D. M., Hildebrandt, P. K., Lewis, G. E., Nyindo, M. B. A., and Ristic, M. (1976): Ultrastructural observations on *Ehrlichia equi* organisms in equine granulocytes. *Infect. Immun.* **13**, 273—280.

Shatkin, A. A., Beskina, S. R., Pankratova, V. N., Popov, V. L., Zakharova, N. A. (1976*a*):

Detection of antigens of *Halprowiae* (Chlamydiae) by the direct immunoperoxidase method (in Russian). *Zh. Mikrobiol.* (*Mosk.*) **1976** (9), 74—78.
Shatkin, A. A., Popov, V. L., Shcherbakova, N. I. (1976*b*): Morphology of *Halprowiae* (Chlamydiae) isolated in Reiter's syndrome (in Russian). *Zh. Mikrobiol.* (*Mosk.*) **1976** (4), 60—64.
Shatkin, A. A., and Popov, V. L. (1982: Current concepts on taxonomy, classification, and nomenclature of Chlamydiae (*Halprowiae*) (in Russian), pp. 90—96. In *Biokhimiya i biophysika mikroorganizmov*, collected papers, issue 10, Gorkiy.
Silverman, D. J., Boese, J. L., and Wisseman, C. L. (1974): Ultrastructural studies of *Rickettsia prowazeki* from louse midgut cells to feces: search for "dormant" forms. *Infect. Immun.* **10**, 257—264.
Silverman, D. J., and Wisseman, C. L., Jr. (1978): Comparative ultrastructural studies on the cell envelopes of *Rickettsia prowazekii*, *Rickettsia rickettsii*, and *Rickettsia tsutsugamushi*. *Infect. Immun.* **21**, 1020—1023.
Silverman, D. J., Wisseman, C. L., Waddell, A. D., and Jones, M. (1978): External layers of *Rickettsia prowazekii* and *Rickettsia rickettsii*: occurence of a slime layer. *Infect. Immun.* **22**, 233—242.
Silverman, D. J., Wisseman, C. L., Jr., and Waddell, A. (1980): In vitro studies of rickettsia--host cell interaction: ultrastructural study of Rickettsia prowazekii-infected chicken embryo fibroblasts. *Infect. Immun.* **29**, 778—790.
Sleytr, U. B. (1978): Regular arrays of macromolecules on bacterial cell walls: structure, chemistry, assembly, and function. *Int. Rev. Cytol.* **53**, 1—64.
Storz, J., and Spears, P. (1977): Chlamydiales: properties, cycle of development, and effect on eukaryotic host cells. *Curr. Top. Microbiol. Immunol.* **76**, 168—214.
Wiebe, M. E., Burton, P. R., and Shankel, D. M. (1972): Isolation and characterization of two cell types of *Coxiella burnetii* phase I. *J. Bact.* **110**, 368—377.
Wisseman, C. L., Jr., and Waddell, A. D. (1975): In vitro studies on rickettsia-host cell interactions: intracellular growth cycle of virulent and attenuated *Rickettsia prowazeki* in chicken embryo cells in slide chamber cultures. *Infect. Immun.* **11**, 1391—1401.