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METABOLISM AND GENETICS OF CHLAMYDIAS AND RICKETTSIAS

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ABSTRACT

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Chlamydial and rickettsial diseases pose a hazard to man and to domesticated and wild animals. The virulence mechanisms which aid the establishment of these obligate intracellular parasites in the eukaryotic host are still not within our grasp. Recent knowledge of the biochemical stratagem, the metabolic capabilities and the genetic diversity of these microbes illustrate fundamental differences in ecology and evolutionary divergence. The preferred site of intracellular residence determines the strategy for uptake, for nutrient assimilation and also for evasion of the host's immunological defenses. The *Chlamydia, Rickettsia,* and *Coxiella* are the most extensively studied of the genera. Whereas the *Ehrlichia* and *Cowdria* are poorly understood, they are also the most intriguing of the Rickettsiae. A number of antigenically and genetically distinct species are identified for the genera *Chlamydia, Rickettsia,* and *Ehrlichia,* whereas the *Coxiella* and *Cowdria* may not represent such a wide diversity. Recent information on the genetic heterogeneity of the chromosomal and plasmid DNAs of the strains of *Coxiella* suggest the diversity is greater than was originally envisioned. New information regarding the antigenic structure of *Cowdria* and their cellular tropisms suggests that they are closely related to the *Ehrlichia*. In this review we compare the metabolic capabilities and the genetic diversity of these different intracellular bacteria.

INTRODUCTION

The microorganisms that constitute the Orders Rickettsiales and Chlamydiales are bacterial parasites of eukaryotic cells. Most of these bacteria are in an obligatory association with the intracellular compartments of host cells (Table 1). Although Rickettsiales comprise a highly heterogeneous group of microorganisms, they differ from Chlamydiales in their association with an arthropod vector, which in some cases is the primary or sole host. Although chlamydia-like organisms have occasionally been described in arhropods, Chlamydia are primarily parasites of mammalian hosts and are transmitted from host to host without the biological intervention of an arthropod vector. This fundamental difference in ecology suggests an early evolutionary divergence. Weisburg, Woese, Dobson & Weiss (1985) compared the ribosomal RNA sequence of a member of the Rickettsiales, *Rochalimaea quintana,* to those of other bacteria and found a specific relatedness to the plant pathogens, agrobacteria and rhizobacteria. Both rickettsiae and plant pathogens are parasites of eukaryotic cells and are transmitted by arthropods, which most likely acted as the bridge in their evolution. A similar study of Chlamydiales (Weisburg, Hatch & Woese, 1986) indicated that these microorganism, although of eubacterial origin, are not closely related to any other group of organisms. A distant relationship was demonstrated to planctomyces, which, as in the case of chlamydiae, have cell walls devoid of peptidoglycan.

The infectious cycle of these intracellular bacteria is initiated at the surface of the host cell. It is from this point forward that distinctions are made between the ability of genera and the species to successfully compete with the host for preferred intracellular sites. Regardless of attempts by the host to eliminate the pathogenic microbes, the different intracellular ecological niches occupied by these bacteria are sustained long enough by the host to provide the requisite environment for their propagation and dissemination to nearby cells. The ability of these bacteria to multiply inside the host is obviously aided by the contents of either the phagosome, the cytoplasm, or the phagolysosome of the eukaryotic cells (Table 2). Many biochemical mechanisms play a role in assuring the intracellular survival of these bacteria. Among these are: (i) the efficiency of uptake, (ii) the pathway to the preferred intracellular site, (iii) the efficiency of competition with the host for nutrients at the site, (iv) the inhibition or utilization of the microbicidal mechanisms at the site, and (v) the mechanisms of exit and journey to new hosts.

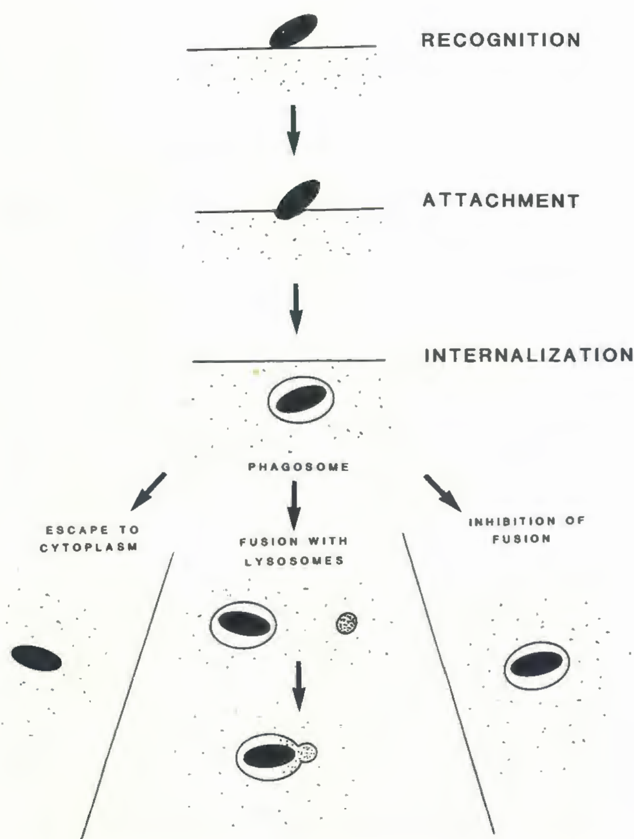

FIG. 1 Schematic illustration of the uptake of obligate intracellular bacteria by eukaryotic cells. The process requires bacterial ligand-host receptor interaction for the recognition, attachment and internalization process. After uptake the bacterium may: (i) escape from the parasitophorous vacuole (phagosome or endosome) to the cytoplasm, (ii) allow fusion of the vacuole with the lysosomes, or (iii) inhibit fusion and remain in the vacuole

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TABLE 1 Some *Chlamydias* and *Rickettsias* pathogenic for humans and animals

Genus[a] species	Intracellular[b] compartment	Disease
Chlamydia	Phagosome	
psittaci		Psittacosis-ornithosis
trachomatis[c]		Trachoma-lymphorgranuloma venereum
Ehrlichia		
sennetsu		Sennetsu rickettsiosis
canis		Canine ehrlichiosis
phagocytophila		Tick-borne fever
equi		Equine ehrlichiosis
risticii		Equine monocytic ehrlichiosis
Cowdria ruminantium		Heartwater
Rickettsia	Cytoplasm	
Spotted fever		
rickettsii		RMSF[d]
conorii		Boutonneuse fever
akari		Rickettsial pox
sibirica		Siberian tick typhus
australis		Queensland tick typhus
Typhus fever		
prowazeki		Epidemic typhus
typhi (mooseri)		Murine typhus
tsutsugamushi		Scrub typhus
Coxiella	Phagolysosome	
burnetii		Q fever

[a] *Cowdria* has not as yet been shown to infect man. *Rochalimaca quintana* (trench fever) is not an obligatory intracellular *Rickettsia*

[b] Preferred intracellular site for the propagation of these bacterial parasites. In the genus *Rickettsia* growth may occur near or in the nucleus of cells infected with the spotted fever group and perinuclearly for *R. tsutsugamushi*

[c] A sexually transmitted disease

[d] RMSF = Rocky Mountain spotted fever

TABLE 2 Adaptations which facilitate the evasion by bacterial pathogens of the microbicidal capacity of the eukaryotic cell

Genus[a]	Host target cell	Special feature	Evasion in:		
			Phagosome	Phagolysosome	Cytoplasm
Chlamydia	Ephithelial	Uptake	Yes	No	No
		Survival	Yes	No	No
		Growth	Yes	No	No
Ehrlichia	Mononuclear	Uptake	Yes	No	No
		Survival	Yes	No	No
		Growth	Yes	No	No
Cowdria	Neutrophil	Uptake	Yes	No	No
		Survival	Yes	No	No
		Growth	Yes	No	No
Rickettsia	Endothelial	Uptake	Yes	No	Yes
		Survival	No	No	Yes
		Growth	No	No	Yes
Coxiella	Macrophage	Uptake	Yes	Yes	No
		Survival	No	Yes	No
		Growth	No	Yes	No

[a] *Cellular* tropism was exhibited by various species of the *Ehrlichia* and the *Cowdria*. Uptake: (i) by mononuclear cells (*E. canis, E. sennetsu, E. risticii*), (ii) by polymorphonuclear granulocytic cells (*E. equi, E. phagocytophila*), and (iii) by endothelial, reticuloendothelial, recently demonstrated in granulocytes (*Cowdria*)

The uptake by the eukaryotic host cell is paramount for the bacteria to gain access to the internal milieu which supports their metabolism and replication. The term "uptake" is used in a general sense and includes the phenomenon of recognition, attachment, and internalization (Fig. 1). The pathways after uptake of these bacteria can be: (i) to lyse the phagosomal membrane thereby exiting the parasitophorous vacuole which is simply referred to as escape, (ii) to prevent phagolysosome fusion so that the ingested microorganism remains within the phagosome, or (iii) to allow the normal phagocytic or receptor-mediated endocytotic process to occur (Hopkins, 1983; Jones, 1980; Mosser & Edelson, 1984; Silverstein & Cohn, 1977).

As pointed out by Moulder (1985), the uptake of bacterial parasites by certain eukaryotic host cells represents significant evolutionary diversity. The uptake and placement of the Chlamydias and Rickettsias into the appropriate intracellular compartment is of interest to us from the view point of the parasites' requirements to carry out specific metabolic exploitation of the host. The biochemical stratagem is the evasion of the microbicidal action of the phagolysosomal milieu. As more information becomes available about the intracellular fate of these bacteria, it becomes apparent that specific modes of uptake into host cells play major roles in determining the final outcome.

A number of antigenically and genetically distinct species are identified for the genera *Chlamydia, Rickettsia* and *Ehrlichia*, whereas the genera *Coxiella* and *Cowdria* may not represent such a wide diversity. More recent information on the genetic heterogeneity of the chromosomal and plasmid DNAs of the strains of *Coxiella* suggest that the diversity is greater than was originally envisioned. Also new information regarding the antigenic structure of *Cowdria* and their cellular

tropisms suggest that they are closely related to the *Ehrlichia*. In this review we will compare the metabolic capabilities and the genetic diversity of these different intracellular bacteria.

The genus *Chlamydia*

Infection by the *Chlamydia* does not require the expenditure of energy by the parasite during the initial steps of uptake (Byrne, 1976; Lee, 1981). However, to prevent phagolysosomal fusion (PLF) the Chlamydiae may require either specific energy metabolism (Hatch, Al-Hossainy & Silverman, 1982) or specific surface components of the infectious elementary body (EB), present at the time of uptake (Hodinka & Wyrick, 1986). Initial internalization and prevention of PLF by Chlamydiae also may involve receptor-mediated endocytotic (RME) mechanisms by the host which preclude PLF (Hodinka & Wyrick, 1986). The EB may be engulfed by professional phagocytes via a pseudopod with formation of the phagosome (classical phagocytosis) or the EB may attach to receptors of the plasma membrane near the microvilli, of non-professional (adsorptive endocytosis) phagocytes, and enter, encased within a vacuolar membrane, the host cell. Acidification (pH < 5,5) of this vacuole may be typical of the phagosome or it may be a receptosome (Pastan & Willingham, 1983), which is also referred to as an endosome (Helenius, Mellman, Wall & Hubbard, 1983). Although the exact mechanism of uptake of Chlamydiae is not known, a new concept of the overall process is thought to be via receptors which preclude PLF (Hodinka & Wyrick, 1986). Hence, in the absence of specific antibodies and/or immune cells, Chlamydiae never enter the host cell phagolysosome. Moreover, the Chlamydiae never enter the host cell cytoplasm where the nutrients required for growth are compartmentalized. Thus, the Chlamydiae must either disrupt the normal acidification process during uptake or they may be activated transiently before reaching their full metabolic capability which seems to be at or near neutral pH (Hackstadt & Williams, 1981a).

The host cell has a primary role in the uptake and internalization of Chlamydiae. Although the uptake process is not inhibited by cytochalasin B (Gregory, Byrne, Gardner & Moulder, 1979; Kuo, 1978a), it is inhibited by cytochalasin D (Ward & Murray, 1984). The discrepancy between the action of these two microfilament polymerization inhibitors may reside in the efficiency of bleb formation by the plasma membrane. Cytochalasin D stimulates more active plasma membrane bleb formation than does cytochalasin B (Meek & Davis, 1986). The evidence of an apparent absence of metabolic role on the part of the parasite is that inhibitors of prokaroytic DNA and protein synthesis do not prevent uptake of the parasite (Kuo, 1978b; Moulder, Hatch, Byrne & Kellogg, 1976).

The intracellular survival of the Chlamydiae depends on the efficiency of circumvention of the phagolysosome. Circumvention is accomplished by specific parasite-directed functions. Evidence for intrinsic structures of *C. psittaci* which prevent the lysosomal fusion response by the host cell were derived from the observations that: (i) viable EBs or EB cell walls do not induce PLF, whereas (ii) heat treated cells and EB cell walls or antibody-coated cells do not inhibit PLF (Eissenberg, Wyrick, Davis & Rumpp, 1983; Levy & Moulder, 1982; Wyrick, Brownridge & Ivins, 1978), and (iii) the viable reticulate bodies (RB) are engulfed and killed by subsequent PLF (Brownridge & Wyrick, 1979). Uptake of large numbers of inactivated or viable Chlamydiae by an internalization process results in destabilization of the host cell plasma membrane and subsequent host cell lysis. Uptake of small numbers of inactivated or antibody-coated EBs result in their destruction by the phagolysosome, whereas viable EBs or their cell walls successfully circumvent this compartment (Friis, 1972; Lawn, Blyth & Taverne, 1973; Todd & Storz, 1975). Although the final site-specific replication is similar, the mechanisms of uptake and cellular tropisms among the Chlamydiae [*C. psittaci*, *C. trachomatis*-lymphogranuloma venereum (LGV), and *C. trachomatis*-trachoma] appear to be quite different (Moulder, 1985).

Investigations designed to answer questions about the relative infectivity versus the *in vitro* metabolic capabilities of the Chlamydiae have concluded that the infectious EBs are less active metabolically than the non-infectious RBs. Some of the metabolic capabilities of the Chlamydiae are summarized in Table 3. While these bacteria do not require energy for their own uptake, they also cannot generate high energy phosphate in the form of ATP via the metabolism of various sugars and amino acids (Weiss, 1965; Weiss & Wilson, 1969). However, they carry out independent: (i) nucleic-acid synthesis via salvage of nucleotides, (ii) protein synthesis via salvage of amino acids, (iii) amino acid and pyruvate metabolism via the Krebs cycle, and (iv) they metabolize exogenously supplied substrates optimally at neutral pH (reviewed in Hatch, Al-Hossainy & Silverman, 1982 & Moulder, 1985).

The fact that the Chlamydiae are neutrophilic indicates that the acid conditions of the phagosome or the endosome may be inhospitable. Thus, the Chlamydiae must disrupt the normal acidification process during the RME process (Hodinka & Wyrick, 1986). Structures of the EB cell wall may serve as ligand and trigger of specific metabolism which could neutralize the acidification process and prevent the delivery of the ligand-receptor to the lysosomes to be degraded (Table 2). Since Chlamydiae are "energy parasites", the vacuolar membrane may be modified, by the parasite, for the uptake of nutrients from the host cell cytoplasm. This function could be carried out either by the insertion of Chlamydial-specific proteins (porins) into the vacuolar membrane or by the diffusion and transport (host function) of metabolites across the vacuolar membrane into the neutralized compartment. Recent investigations into the physiological changes that occur by specific treatments *in vitro* indicate that major outer membrane protein (MOMP) of Chlamydiae serves as a porin (Bavoil, Ohlin & Schachter, 1984). Although the MOMP plays a role in the structural rigidity of the cell wall, it serves as a porin and is activated by the breaking of disulfide bonds (adjacent cysteines). Thus, the EB outer membrane is impermeable to hydrophilic solutes, but after uptake it is exposed to reducing conditions (of the endosome or phagosome) which transform the metabolically inactive EB into a metabolically active RB (Bavoil, Ohlin *et al.*, 1984). A delicate balance of nutrients and reducing equivalents must be maintained during the replication phase of the developmental cycle. Any imbalance of this environment probably induces the formation of the EB which completes the cycle of development. Release of the EB initiates a new round of infection in nearby eukaryotic cells.

The limited genetic diversity in natural isolates of Chlamydiae is evidenced by the division of the genus into two species, *C. trachomatis* and *C. psittaci* (Kingsbury & Weiss, 1968; Weiss, Schramek, Wilson & Newman, 1970). *Chlamydia trachomatis* is further divided into three biovars: trachoma, LGV and mouse. Trachoma and LGV infect humans while the mouse biovar does not. The trachoma and LGV biovar are related antigenically and genetically while the mouse biovar does not share this relationship. The natural hosts for *C. psittaci* are birds and lower mammals while humans are

TABLE 3 Biochemical mechanisms characteristic of chlamydia and rickettsial parasite-specific multiplication

Metabolic parameter[a]	*Clamydia*	*Ehrlichia*	*Cowdria*	*Rickettsia*	*Coxiella*
Energy dependent uptake	No	Unk	Unk	Yes	No
ATP from oxidation of glucose and glutamate	No	Unk	Unk	Yes	Yes
Independent nucleic-acid synthesis	Yes	Unk	Unk	Yes	Yes
de novo	No			No	Yes
salvage	Yes			Yes	Yes
base	No			No	Yes
nucleoside	No			No	Yes
nucleotide	Yes			Yes	No
Independent protein synthesis	Yes	Yes	Yes	Yes	Yes
de novo	Unk	Unk	Unk	Unk	Yes
salvage	Yes	Unk	Unk	Yes	Yes
Carbohydrate metabolism	Yes	Yes	Unk	Yes	Yes
glycloysis	No	Unk		No	Yes
gluconeogenesis	No	Unk		No	Yes
Amino acid and pyruvate metabolism					
Krebs cycle	Yes	Yes	Unk	Yes	Yes
Lipid metabolism[b]	Yes	Unk	Unk	Unk	Yes
de novo	Yes			Unk	Yes
salvage	Yes			Unk	Unk
Neutrophile	Yes	Yes	Yes	Yes	No
Acidophile	No	No	No	No	Yes

[a] Selected enzymes, metabolic intermediates or final products were utilized by the different Rickettsias

[b] Unpublished results indicate that isotopically labeled glucose and glutamate were detected in lipid molecules of *Coxiella* (J. C. Williams & T. Hackstadt), and Gaugler, Neptune, Adams, Sallee, Weiss & Wilson, 1969, demonstrated lipid synthesis in *Chlamydia*

incidentally infected. The molar % G+C of DNA is 44,0; 41,9; 42,9 and 41,3 for trachoma, LGV, mouse and *C. psittaci*, respectively. Plasmids of 4×10^6 daltons have been identified for all but the mouse biovar (Hypiä, Larsen, Stihlberg & Terho, 1984; Joseph, Nano, Garon & Caldwell, 1986; McClenanghan, Herring & Aitken, 1984), but their role in virulence or metabolism by Chlamydiae has not been determined. The genome size among the Chlamydiae is 4 to 6×10^8 daltons. The degree of DNA homology is 100 % between the trachoma and LGV biovars, while the mouse and *C. psittaci* are only 30 to 60 % and 10 % homologous with the former two biovars. A genus-specific lipopolysaccharide (LPS) antigen has been identified using a monoclonal antibody against the LPS fraction of Chlamydiae (Caldwell & Hitchcock, 1984). The role of Chlamydial LPS in virulence has yet to be determined. This common determinant is shared by all Chlamydiae, even though much diversity in both antigenic structure (Caldwell, Kuo & Kenny, 1975; MacDonald, 1985) and genetic composition (Moulder, 1984; Petersen & De la Maza, 1983) exist between the Chlamydiae species. Diversity in antigenic structure also was attributed to the MOMP (see above). This surface antigen can induce antibody capable of neutralizing infectivity *in vitro* (Caldwell & Perry, 1982) and contains epitopes which contribute to the species, sub-species, and type-specific definition of the 15 known serovars of this species (Caldwell & Schachter, 1982). Recently, genetic diversity has been probed by molecular cloning of the MOMP of *C. trachomatis* (Allan, Cunningham & Lovett, 1984; Nano, Barstad, Mayer, Coligan & Caldwell, 1985). Since little antigenic (Caldwell & Judd, 1982) or structural relatedness between the MOMPs of *C. trachomatis* and *C. psittaci* was demonstrated, molecular cloning should resolve the question of conservation of functional polypeptide regions perhaps associated with the membrane.

The genus *Rickettsia*

During infection the requirement for the expenditure of energy for uptake and escape to the cytoplasm has been well documented for the typhus biotypes (Austin & Winkler, 1987; Winkler, 1986). The energy-dependent uptake process involves ligand recognition of a complimentary host cell receptor to which the Rickettsiae are irreversibly adsorbed (Ramm & Winkler, 1973). Although the receptor on the red cell contains cholesterol (Ramm & Winkler, 1976), the receptor on the plasma membrane of nucleated cells is not known (Walker, Firth, Ballard & Hegarty, 1983; Walker, 1984; Walker & Winkler, 1978). The receptor hypothesis has been proven for only the typhus group. The Rocky Mountain spotted fever (RMSF) and scrub typhus groups may have similar energy requirements, but the requirement for a ligand-receptor interaction has not been studied adequately to distinguish between the groups. Active participation by both Rickettsiae and the host cells is required for uptake to occur by induced phagocytosis (Walker & Winkler, 1978).

Internalization of adsorbed Rickettsiae requires active participation of the host cell. Treatment of host cell with heat (Cohn, Bozeman, Campbell, Humphries & Sawyer, 1959), energy poisons (i.e., N-ethylmaleimide, fluoride), and cytochalasin B inhibits the uptake of Rickettsiae (Walker & Winkler, 1978). However, inhibitors of DNA replication (i.e., ultraviolet light, x-irradiation), of microtubule polymerization (i.e., colchicine), and of depolymerization of microtubules (i.e., taxol) do not inhibit the internalization of the Rickettsiae (Austin & Winkler, 1987; Cohn *et al.*, 1959).

Professional phagocytes can engulf live or killed Rickettsiae, but the nonprofessional phagocytes require active participation of both parties. Uptake by induced phagocytosis appears to be the method of choice for the survival and growth of the parasite. Parasite-specific antibody facilitates uptake and inhibition of growth of Rickettsiae. Thus, an additional role for antibody may be the prevention of the action of phospholipase A on the host phagosomal membrane. The participation of phospholipase A in the escape strategy of the parasite to the cytoplasm was elaborately studied (Austin & Winkler 1987; Winkler, 1982). Opsonization of Rickettsiae, in deference to induced phagocytosis, channels the Rickettsiae away from the escape strategy (the phospholipase A exit phenomenon) into an Fc-mediated channeling to fusion with the lysosomal vacuole (Brewer, Harvey, Mayhew & Simpsom, 1984) which results in the destruction of the parasite.

The participation of phospholipase A in the ligand-receptor recognition step is not clearly understood (Winkler & Miller, 1982). Compounds which inhibit hemolysis by inhibiting adsorption to the red blood cell also prevent the release of free fatty acids (Winkler & Miller, 1984). When the phospholipase A is activated it specifically releases host free fatty acids and lysophosphatides with concomitant loss of host cell membrane integrity. Thus, one assumes that the phenomenon of rickettsial toxicity is due to the massive invasion of the host cell by Rickettsiae causing cell lysis.

Rickettsiae which have adapted mechanisms of escape from the phagosomal vacuole into the cytoplasm are the typhus, scrub typhus, and spotted fever groups (Table 2). The intracellular survival of the Rickettsiae depends on the efficiency with which the actively metabolizing bacterium escapes the confines of the phagosome. Escape of Rickettsiae from the phagosome is very rapid in all eukaryotic cells examined (Andrese & Wisseman, 1971; Ewing, Takeuchi, Shirai & Osterman, 1978; Rikihisa & Ito, 1980; 1982; Winkler, 1986; Winkler & Miller, 1982). The escape strategy is that of actively digesting the phagosomal membrane with the aid of a phospholipase A enzyme which may or may not be derived from the parasite (see above). Upon arrival in the substrate rich cytoplasm, the competition with the host for specific metabolites is usually won by the Rickettsiae. The substrates for the generation of energy and the synthesis of macromolecules via the major metabolic pathways are satisfied by both orthodox and unorthodox pathways (Table 3). The most important adaptation for the Rickettsiae to the intracytoplasmic environment is the transport and exhange of high energy phosphates in the form of purine and pyrimidine nucleotide (mono-, di- and triphosphates) (Winkler, 1976; Williams & Peterson, 1976; Smith & Winkler, 1977; Williams, 1980; Atkinson & Winkler, 1985; Winkler & Daugherty, 1984a; 1986). Recently, the importance of the uptake of uridine 5′-diphosphoglucose in providing a source of glucose for rickettsial macromolecular synthesis was demonstrated (Winkler & Daugherty, 1986). Protein synthesis appears to be conducted after the transport of amino acids (Smith & Winkler, 1977; Winkler & Daugherty, 1984b; Zahorchak & Winkler, 1983). The extent to which the Rickettsiae synthesize their own amino acids is not known. Recent studies (Austin, Turco & Winkler, 1987) indicate that sufficient concentrations of intracytoplasmic serine and glycine are required to support the growth of *R. prowazekii*. When these amino acids are limiting, the host cell can grow but the Rickettsiae cannot compete effectively for the limiting soluble pools. The importance of the intracellular potassium cation levels has been demonstrated by studying rickettsial macromolecular synthesis *in vitro* (Bovarnick & Schneider, 1960; Bovarnick, Schneider & Walter, 1959; Winkler, 1984). High potassium and low sodium concentrations were shown to be optimal for the transport of substrates and for the synthesis of macromolecules. Host functions such as protein synthesis (Turco & Winkler, 1983; Weiss, Newman, Grays & Green, 1972), nucleic acid synthesis (Weiss & Dressler, 1958; Wisseman, Waddell & Walsh, 1974; Wisseman & Waddell, 1975; Wisseman, Edlinger, Waddell & Jones, 1976; Turco & Winkler, 1982) and celldivision (Bozeman, Hopps, Danauskas, Jackson & Smadel, 1956; Hopps, Jackson, Danauskas & Smadel, 1959) are not required for the replication of Rickettsiae. Growth of the Rickettsiae also does not require the nucleus of the eukrayotic cell (Stork & Wisseman, 1976). Rickettsiae multiply best in a host that is providing free soluble pools of substrates for the parasite.

Genetic diversity in natural isolates of Rickettsiae is evidenced by the division of the genus into three groups (Weiss & Moulder, 1984). The three groups are the typhus (with 3 species), the spotted fever (with 11 species), and the scrub typhus (with a single species and three serovars). The natural arthropod hosts are the louse *(R. prowazekii)*, the flea *(R. typhi)*, the tick [*R. canada, R. rickettsii, R. conorii, R. parkeri, R. australis, R. montana, R. rhipicephali, R. helvetica* (Peter, Williams & Burgdorfer, 1985), *R. sibirica, R. slovaca*] and the mite *(R. akari, R. tsutsugamushi)*. The molar % G+C of DNA is 29–30 (typhus group), 32–33 (spotted fever group), and is unknown for the scrub typhus group with a genome size of 13 to 15 $\times$ 10^8 daltons. Restriction endonuclease analysis of rickettsial genomic DNAs has provided a means for the establishment of intraspecies identity of typhus rickettsial isolates (Regnery, Tzianabos, Esposito & McDade, 1983; Wood, Sikorshki, Atkinson, Krause & Winkler, 1984). Differences in polypeptide profiles among various rickettsial isolates were also detected (Dasch, Samms & Weiss, 1978). The identification of unique restriction fragment length polymorphisms (RFLP) and the subcloning of these specific DNA fragments for use as probes have led to the further characterization of *R. prowazekii* isolates. Indeed, *R. prowazekii* isolates from southern (USA) flying squirrels were readily differentiated from human isolates of *R. prowazekii* from Europe and Africa (Regnery, Fu & Spruill, 1986).

The expression (biosynthesis of a polypeptide) in *Escherichia coli* of cloned rickettsial genomic DNAs has been accomplished by several investigators. Evidence for the expression of fully functional rickettsial enzymes in the *E. coli* host was first demonstrated by complementation analysis of the rickettsial citrate synthase gene (Wood, Atkinson, Sikorski & Winkler, 1983) (Table 4). The strategy for isolating the clone was to utilize an *E. coli* host that was defective for glt A. Transformants were selected on a medium which could not support the growth of the host in the absence of a functional citrate synthase. The regulation of partially purified citrate synthase of *R. prowazekii* is distinguishable from that of free living gram negative bacteria and the eukaryotic host (Phibbs & Winkler, 1982). The *E. coli* enzyme is strongly inhibited by alphaketoglutarate, whereas the rickettsial enzyme responds negatively to ATP. The cloned gene in *E. coli* still retained the rickettsial control pattern, but the *in vivo* expression of citrate synthase was not sufficient to restore full growth potential. The role of metabolic regulation or other signals responsible for this phenomenon is unclear. Characterization of the ADP/ATP translocator for *R. prowazekii* (Winkler, 1976) led to the cloning and expression of this transport system, unique for rickettsial physiology, in *E. coli* (Krause, Winkler & Wood, 1985). The cloned rickettsial ADP/ATP translocator exhibited the same characteristics as the native translocator in *R. prowazekii*.

The genus *Coxiella*

The uptake of *Coxiella* by host cells has not been adequately studied for a detailed description of the steps involved in the recognition, attachment, and entry processes (Fig. 1). Expenditure of energy during the uptake process is not a requirement since *Coxiella* cells do not metabolize exogenously supplied substrates as neutral pH (Hackstadt & Williams, 1981). *Coxiella* cells in phase I (virulent, smooth LPS) are poorly adsorbed by both professional and nonprofessional phagocytes, whereas cells in phase II (avirulent rough LPS) are taken-up more readily (Burton, Kardova & Paretsky, 1971; Kazar, Brezina, Schramek, Urolgyi, Pospisil & Kovacova, 1974; Kazar, Skultetyova & Brezina, 1975; Kishimoto & Walker, 1976; Ormsbee, Peacock, Gerloff,

TABLE 4 Genetic correlations of virulence in *Coxiella burnetii*

Strain	Chro DNA deletion	Plasmid prototype	LPS	Suppressive complex
Nine Mile I	No	QpHI	S	Yes
Nine Mile II	d1A*	QpHI	R	No
RSA 514	d1B*	QpHI	Semi-R	Yes
Australian	No	QpHI	S and R	No
K Q154	No	QpRS	S and R	No
P Q173	No	QpRS	S and R	No

* d1A—18 kilobase deletion

* d1B—29 kilobase deletion with a common terminus to d1A

TABLE 5 Plasmids of *Coxiella burnetii*

Isolate	Origin	Disease	Plasmid type
Nine Mile, phase I	Tick, Montana	Acute	QpHI
Nine Mile, phase II	Egg passage	None	QpHI
RSA 514	Egg passage	Acute	QpHI
M-44	Human blood, Greece	Acute	QpHI
Henzerling	Human blood, Italy	Acute	QpHI
Australian	Unknown	Acute	QpHI
Scottish	Sheep	Acute	QpHI
Cypriot	Sheep	Acute	QpHI
KQ154	Human heart valve	Endocardits	QpRS
PQ173	Human heart valve	Endocartidis	QpRS
MSU Pricilla Q177	Goat	Abortion	QpRS
Canada Q218	Goat	Abortion	QpRS

Tallent & Wike, 1978; Wisseman, Fiset & Ormsbee, 1967). Opsonization also facilitates the uptake and placement of *Coxiella* in the phagolysosome where immuno-competent cells may have an advantage in killing this acidophilic bacterium (Hackstadt & Williams, 1981) (Table 2).

Previous studies by others (reviewed in Baca & Paretsky, 1983) indicate that uptake into host cells is considered to be a passive event on the part of the parasite. We (C. O. Kindmark and J. C. Williams) believed that this conclusion was potentially incorrect because of the efficiency with which *Coxiella* infect animals and some eukaryotic cells in tissue culture in the presence of serum. The common component in all of the *in vitro* culture systems is the presence of serum which could contain components which interact with *Coxiella* and the cultured cells. In the intact animal there is an obvious advantage for a parasite which can use the host's natural defense mechanisms to establish infection. Therefore, we initiated studies of the interaction of *Coxiella* with the acute phase reactants (APR) (Kushner, 1982). Recent studies on the interaction of phase I *Coxiella* with non-immune immunoglobulin (Williams, Thomas & Peacock, 1986), C-reactive protein (CRP), complement components (C), and ceruloplasmin (CP) indicate that the APRs may play a major role in facilitating the uptake of *Coxiella* (C. O. Kindmark and J. C. Williams, unpublished results). The binding (determined by microimmunofluoresence) of immunoglobulin, CPR, C3, C4, C8, and CP to the surface of *Coxiella* was both avid and specific (unpublished observations). Furthermore, the viability of *Coxiella* was not reduced after attachment of these components to the cell surface. Thus, we propose that the uptake of *Coxiella* is facilitated by APR proteins, by host cells carrying the appropriate receptor. This mechanism of uptake is both expeditious and unique. The fact that CP is bound by *Coxiella* suggests that Q fever hepatitis may be enhanced via the asialoglycoprotein receptor on hepatocytes (Steer & Ashwell, 1980). Therefore, the mechanisms which are usually designed to prevent infection of the host by unwanted parasites may actually facilitate the uptake and placement of *Coxiella* in the phagolysosome of target cells. These interactions imply that the molecular mechanisms involved are specific and that the density of the *Coxiella* ligand-APR receptors and their specificity ensure that both active and passive modes of uptake may be operative.

The ability of *Coxiella* to take advantage of the intraphagosomal milieu was first indicated by *in vitro* experiments which demonstrated that the transport of substrates across the cell wall is activated by decreasing the pH (<5,5) of the medium to conditions approximating the acid conditions of the phagolysosomes (Hackstadt & Williams, 1981a). This hypothesis was confirmed by increasing the pH of acidic vacuoles by adding lysosomotropic drugs to *Coxiella*-infected eukaryotic cells to prevent replication of the parasite. The transport of certain substrates and their metabolism induced the production of ATP (Hackstadt & Williams, 1981b). Prior to these studies numerous cytoplasmically located *Coxiella* enzymes and pathways were discovered (for review see Thompson, 1987). The different metabolic capabilities of *Coxiella* are summarized in Table 3. Thus, acidification of the parasite's phagosome is the necessary event for *Coxiella* to initiate active metabolism and subsequent multiplication.

Infection of the eukaryotic cell may occur by all members of the developmental cycle (McCaul & Williams, 1981), since a one to one correlation between the number of *Coxiella* cells, the plaque forming units, and the mouse infecting particles was shown (Williams, Peacock & McCaul, 1981). Morphological variants produced during the multiplication of *Coxiella* in the phagolysosome have been characterized as large cell variants (LCV), small cell variants (SCV), and endospores, with the LCV and SCV predominating (McCaul & Williams, 1981). These morphological variants metabolize exogenously provided substrates with different efficiencies (Hackstadt & Williams, 1981c; McCaul, Hackstadt & Williams, 1981). The LCV is the most efficient and the SCVs which survive physical abuse, such as sonication and pressure treatment (McCaul *et al.*, 1981), metabolize less well. The intraphagosomal events necessary for the production of the different morphologic forms have not been discovered. We assume that the initial conditions of the infected phagolysosome or an imbalance in them (whatever they may be) trigger the different stages of the developmental cycle (McCaul & Williams, 1981).

The regulatory signals which trigger the different stages of the morphologic variants are unknown.

Limited genetic diversity in natural isolates of *Coxiella* is indicated by the provision of only one genus and one species, *C. burnetii* (Weiss & Moulder, 1984). The molar % G+C of DNA is 43 and the genome size is 11×10^8 daltons (Myers, Baca & Wisseman, 1980). The relationship of *Coxiella* to other members of the tribe *Rickettsiae* is vague. *Coxiella* may not share any phylogenetic relationships with the Rickettsiae. Antigenic variation among intrastrain isolates is genetically determined as evidenced by marked differences in the expression of surface proteins (Williams, Johnston, Peacock, Thomas, Stewart & Portis, 1984; Williams & Stewart, 1984) and by the apparent mutational variation in the LPS structure of both intrastrain and interstrain isolates (Hackstadt, Peacock, Hitchcock & Cole, 1985; Hackstadt, 1986; Amano, Williams, Missler & Reinhold, 1987). The phase variation described for *Coxiella* appears to be unique among the Rickettsias (Stoker & Fiset, 1956). Appropriate monoclonal antibodies can rapidly distinguish certain strains of *Coxiella* (Williams, *et al.*, 1984). The monoclonal antibodies appear to recognize either epitopes on polypeptides that now appear on the surface in the avirulent organism or sugar moieties on the LPS present in the virulent organism but not in the attenuated strain.

Restriction endonuclease analysis of *Coxiella* genomic (Vodkin, Williams & Stephenson, 1986; Vodkin & Williams, 1986) and plasmid (Samuel, Frazier, Kahn, Thomashow & Mallavia, 1983; Vodkin *et al.*, 1986; Vodkin & Williams, 1986) DNAs has revealed marked RFLPs. These genetic alterations undoubtedly are related to the observed disease entities which range from acute to chronic dispositions (Peacock, Philip, Williams & Faulkner, 1983). Moreover, recent studies have obtained genetic correlations of virulence in both the RFLPs of the chromosomal and plasmid DNAs (Table 4 and 5). Common laboratory and vaccine strains carry 38 kb plasmid (Samuel *et al.*, 1983). Strains that caused hepatitis or endocarditis in humans or abortions in goats have a 41 kb plasmid (Vodkin *et al.*, 1986; Vodkin & Williams, 1986). These plasmids are related but they were not derived from each other by any simple recombination process. Thus, the RFLPs associated with the plasmids correlate well with an altered spectrum of pathology.

Other virulence properties which correlate with the above described RFLPs have been described. An immunomodulatory activity (IMA) which induces either nonspecific enhancement or immunosuppression has been identified (Damrow, Williams & Waag, 1985; Williams & Cantrell, 1982; Williams, Damrow, Waag & Amano, 1986). Individual components of IMA which play a role in antigen-specific immunosuppression were recently described (Waag & Williams, submitted). The structure of the phase I IMA is composed of at least three components in an obligatory association on the cell surface and interdependent for their biological activity. Genetic correlations between the biosynthesis of LPS and components of the IMA were predicted based on the identification of inter- and intrastrain variations in both the structure of LPS and the expression of the IMA (Vodkin & Williams, 1986; Waag & Williams, submitted).

The genus *Ehrlichia*

The leukocytic Rickettsiae which parasitize circulating leukocytes of humans and a variety of other animals are internalized by cells of the mononuclear and granulocytic series (Ristic, 1986). This very interesting group of microorganisms is difficult to propagate *in vitro* and the various species were only recently recognized (Ristic & Huxsoll, 1984). Although it is known that these bacterial parasites replicate in the phagosomal vacuole (Table 1), the steps involved in the uptake processes are not known (Table 2).

A special cellular tropism may facilitate the parasitism of specific cells of the reticuloendothelial system (RES). The Ehrlichiae probably exert their cellular tropism at the level of receptor interactions with specificities residing with both the bacterium and the host cell. No adequate studies have been performed to determine these cellular interactions at the level of molecules. Differential specificities exhibited by the Ehrlichiae are: *E. canis* infects canine monocytes, lymphocytes and rarely neutrophils; *E. phagocytophila* infects ovine, bovine, and cervine neutrophils, eosinophils, basophils, and monocytes; *E. equi* infects equine and canine granulocytes; *E. sennetsu* infects human mononuclear cells, and *E. risticii* infects equine granulocytes (Ristic, 1986).

Since the Ehrlichiae apparently replicate in the phagosomal vacuole and carry out a developmental cycle similar but not identical to that of the Chlamydiae (Ristic & Huxsoll, 1984), these bacteria may have similar energy and metabolic requirements (Table 3). We cannot assume that the uptake mechanisms are similar to those of the Rickettsiae or Chlamydiae because obvious differences in their cellular tropisms exist. Thorough studies of the interactions of Ehrlichiae with the surfaces of the different eukaryotic cells are required before the molecular mechanisms governing the tropism can be resolved. The metabolic potential of the Ehrlichiae has recently been studied by Weiss *et al.* (1985a,b). They verified that the organisms metabolize glutamine optimally at neutral pH. This is consistent with their growth in the phagosomal vacuole. The mechanism of uptake and inhibition of PLF is unknown.

Genetic diversity in natural isolates of Ehrlichiae is evidenced by the division of the genus into 5 species (Ristic & Huxsoll, 1984). That the tick is the only vector for all of the species has not been confirmed (Ristic, 1986). These microorganisms are difficult to propagate in cell cultures. They have been successfully cultivated in several primary blood monocyte lines (Ristic, 1986). Continuous cultivation in endothelial and murine macrophage cell lines may be the most effective system for most of the Ehrlichiae (Cole, Ristic, Lewis & Rapmund, 1985). The natural hosts for the Ehrlichiae demonstrate their wide diversity. The molar % G+C of DNA, the genome size, the presence of plasmids, and the degree of DNA homology with other Rickettsiae are unknown. Therefore, the diversity in antigenic structure is determined serologically by using the indirect fluorescent antibody test (Ristic, Huxsoll, Weisiger, Hildebrandt & Nyindo, 1972). Information concerning the pertinent genetic characteristics of the leukocytic Rickettsiae should be rapidly expanded in the near future now that continuous *in vitro* propagation has been achieved.

The genus *Cowdria*

Cowdria ruminantium, the etiologic agent of heartwater, parasitizes vascular endothelial cells and primarily neutrophils in the circulating blood (Logan, Whyard, Quintero & Mebus, 1987). The recent discovery that the microorganism can be successfully grown in calf endothelial cells *in vitro* will facilitate future studies (Bezuidenhout, Paterson & Barnard, 1985). The mechanism of uptake of *Cowdria* has not been studied, but the microorganism has only been observed in the phagosomal vacuole.

The developmental cycle of *Cowdria* appears to be very similar to that of the Ehrlichiae (Prozesky, 1987). Since the microorganism multiplies in the phagosomal

vacuole, it is probably a neutrophilic bacterium. Like the Ehrlichiae it demonstrates an affinity for a particular cell type of the RES, but the cell type which *Cowdria* parasitizes is clearly different from those parasitized by the Ehrlichiae (Ristic, 1986). Thus, *Cowdria* should possess specific molecular structures which serve to facilitate uptake by the neutrophil cells. This unique association of *Cowdria* with the neutrophil cell also suggests that some specific metabolic function of the host is required for growth, or that other cells of the RES series are better armed to inactivate *Cowdria*.

Natural diversity of *Cowdria* is indicated by the identification of antigenically related but clearly different strains. Recent studies show that 4 isolates (Kwanyanga, Kümm, Gardel and Mali) of *Cowdria* and one member of the (*E. equi*) Ehrlichiae share antigens (Holland, Logan, Mebus & Ristic, 1986; Logan, Holland, Mebus & Ristic, 1986). Serologic cross-reactions were not observed between *Cowdria, E. senetsu, E. risticii*, or with12 Rickettsiae, including *C. burnetii*. This finding is particularly disturbing since equine ehrlichiosis has been recognized to occur in the United States (Madewell & Gribble, 1982; Brewer *et al.*, 1984). Thus, it is possible that *Cowdria* species already exist in the United States and their antigenic relatedness with *E. equi* has mistakenly lead to the diagnosis of equine ehrlichiosis.

In order to better understand *Cowdria* and to devise strategies to combat its devastating effects in domestic herds, genetic analysis should be performed on various functions. Since *Cowdria* can be grown in tissue culture, rapid advances should be made in describing antigenic variation, pathogenecity, host range, and tropism with the aim of developing diagnostic reagents and effective vaccines. A particularly interesting feature of the pathogenesis of *Cowdria* is the propensity to infect the brain. This observation alone would suggest that *Cowdria* possesses unique virulence factors which may not be associated with other Rickettsiae. Such studies will enhance our ability to understand the biochemical mechanisms underlying the metabolism, genetics, and virulence of these important pathogens.

PROSPECTS

This review has presented a brief summary of a field in a state of transition. Recently our knowledge of the biochemical mechanisms and the metabolic capabilities of these obligate intracellular parasites has advanced. We are only now beginning to study the genetic diversity which is responsible for the unique properties of these microbes. *Chlamydia, Rickettsia*, and *Coxiella* are the most extensively studied of the genera. Whereas the Ehrichiae and *Cowdria* are poorly understood, they are also the most intriguing of the Rickettsiae. Although several major problems remain unsolved, our understanding of the biological systems has improved. The future is bright and we should be able to establish research priorities.

The rickettsial and chlamydial diseases pose a hazard to both man and domesticated and wild animals. The virulence mechanisms which aid the establishment of these parasites in the host are still not within our grasp. More progress has been made with the Chlamydiae and Rickettsiae affecting humans than those primarily of veterinary interest. However, substantial uncertainty exists regarding the basic interactions of these parasites with host defenses. To understand the success of these intracellular parasites more thorough studies of the metabolic capabilities should be conducted. At first this approach may seem only academic; but if we are going to be successful in devising efficacious vaccines the target must be well defined. The appropriate subunit vaccine might very well be a transport protein rather than a purely structural protein. Opsonization directed by protein-specific antibodies which neutralize the metabolic capabilities of these parasites will undoubtedly control their ability to perform evasive action during the uptake process.

Since most of these bacteria undergo developmental changes during the infectious process, it is necessary for us to study intensively the molecular genetics of antigenic variation of the individual members of the various developmental cycles. We have only recently learned that members of the developmental cycle of *Coxiella* synthesize different surface antigens (T. F. McCaul & J. C. Williams, unpublished data). It is also clear that some hosts do not mount an immune response to certain of the very resistant morphological forms of *Coxiella*. Detailed chemical comparisons of the virulent and avirulent strains' cell walls, OMPs, LPSs and ligand-receptor protein interactions (especially those exhibiting tropism) are necessary to determine which antigens may be used for stimulating a protective cellular immune response.

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