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SELECTED OBSERVATIONS ON RICKETTSIAE AND THEIR HOST CELLS*

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Summary. — The study of the interaction between rickettsiae and their host cells is in its infancy. Members of the genera *Rickettsia*, *Coxiella* and *Rochalimaea* show considerable diversity in host cell range (in vivo vs. in vitro), kind of association with host cell (pericellular, intracellular), mode of entry, interactions with various host cell membranes, intracellular localization (intraphagosomal, free in cytoplasm, intranuclear), adaptation to preferred microhabitat (e.g., optimal pH for enzymes), details of growth cycle, mechanisms of host cell damage. Quantitative in vitro methods exist for the study of infection cycles. Knowledge of nutritional requirements is almost non-existent. Host factors, (e.g., antibody, lymphokines, immune interferon) influence intracellular rickettsiae. Rickettsia-host cell interactions remain a fertile field for discovery.

Key words: rickettsiae; host cells; localization; immune response

Introduction

In the last few years there has been a surge in information relevant to the association between those organisms that we conventionally consider as "rickettsiae" and their host cells and a few areas of clarification are beginning to appear. For the most part, however, we are still in that awkward phase of seemingly increasing complexity that precedes sweeping simplification by broad unifying principles. The restricted array of organisms so conveniently considered collectively in the past as "rickettsial agents of human disease" is in fact now known to be composed of organisms which show enormous diversity of properties, actions and host cell associations and which may actually be of diverse origins showing both convergent and divergent evolution — e.g., *Rochalimaea*, *Rickettsia* and *Coxiella* (Wisseman, 1981, 1983). It is becoming increasingly difficult, awkward and a bit tedious to generalize and to consider them all briefly in any brief review because of the many qualifications necessary for scientific accuracy.

This review will of necessity present a highly selected sampling of the available literature, with no criticism implied with respect to the extensive

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excellent work not included. For example, although arthropods are an integral part of the natural history of "rickettsiae", we have been forced to ignore entirely the interactions between rickettsiae and arthropod cells.

This review has been biased towards a description of the infection cycles of the various rickettsiae and simple quantitative methods now available for their study. Such quantitative methods were introduced in the late 1950's by Smadel's group at the Walter Reed Army Institute of Research (Bozeman *et al.*, 1956; Schaechter *et al.*, 1957; Cohn *et al.*, 1959; Hopps *et al.*, 1959*a*, *b*). Subsequent advances in tissue culture methods, in quantitation of rickettsiae by plaque counts (Wike *et al.*, 1972), in methods for counting the absolute number of rickettsial bodies in a suspension (Silverman *et al.*, 1979) and in preparing highly purified rickettsial suspensions greatly enriched in viable organisms (Hanson *et al.*, 1981) prompted us in the early 1970's to begin to refine the methods for quantitative studies on rickettsia-host cell interactions and to describe some of the basic features of rickettsial growth (Wisseman and Waddell, 1975; Wisseman *et al.*, 1976*a*, *b*; Stork and Wisseman, 1976; Silverman and Wisseman, 1978, 1979; Silverman *et al.*, 1978, 1980, 1981). Such quantitative methods, easily incorporated in the daily laboratory routine, coupled with the modern approaches of cell and molecular biology and ultrastructural methods promise to yield much information on the basic biology of rickettsiae. The following review stresses the quantitative approach and considers selected findings in this general framework.

Infection cycle

Our systematic studies of rickettsial infection of cells in culture led us to define the *infection cycle* of *Rickettsia* spp. in cell cultures to include (1) entry of the organism into the host cell ("uptake"), (2) the intracellular growth cycle and (3) exit or escape from the host cell. We have developed quantitative methods for the study of at least the entry phase and the intracellular growth cycle, with some qualitative observations on the escape phase (Wisseman and Waddell, 1975). Here we describe briefly the methods and attempt to analyze selected observations by us and others in the context of the infection cycle concept. Some of the work described very briefly below, especially the original quantitative work on uptake systems, has not yet been published in full detail, even though the principles have been applied on a daily basis in our laboratories for some years and have been shared with all who have come to our laboratories. *Rickettsia prowazekii* and *R. rickettsii* have been studied most intensively, but the general principles have been found to apply to other members of the genus *Rickettsia* — i.e., *R. mooseri*, *R. tsutsugamushi*, *R. canada* and members of the spotted fever group.

Host cell range: in vivo vs. in vitro

There is an apparent disparity between the host cell range of rickettsiae in vivo in the intact mammal and in cell cultures in vitro. In man and animals infected by a „natural" peripheral route, to the extent that it is known,

members of the genus *Rickettsia* show a unique tropism for vascular endothelial cells ("target cell") and, in the case of *R. rickettsii*, also for adjacent vascular smooth muscle cells (Wohlbach, 1948; Walker *et al.*, 1977; Walker and Cain, 1978; Wisseman, 1981). Although rickettsiae may be found to some extent in other cells in vivo, e.g. circulating blood-borne monocytes (Bukles *et al.*, 1975; Deshazo *et al.*, 1976), they are rare even in the mononuclear cells that comprise the typical perivascular immuno-inflammatory response surrounding the sites of endothelial cell infection (Wohlbach, 1948; Walker and Cain, 1978; Wisseman, 1981; Todd *et al.*, 1984). The factors operative in this apparent, severe in vivo target cell restriction of infection by *Rickettsia spp.* are unknown, for other types of cells do become infected when the organisms are artificially introduced into other sites — e.g., mesothelial cells after intraperitoneal inoculation and alveolar cells after infection of the lungs by the intranasal route. *Coxiella burnetii* appears to infect preferentially histiocytes, macrophages and Kupfer cells (Wohlbach, 1948; Wisseman, 1981), though infection of hepatocytes has been reported following intraperitoneal inoculation. This restriction of target cells for *C. burnetii* within a multicellular host may reflect a selection imposed by the passive endocytosis-dependent entry mechanism (see below).

In contrast to the restricted host cell range in vivo, in cell culture *Rickettsia* spp. infect and grow in a broad range of eukaryotic cell types of protozoan, arthropod, fish, reptilian, avian and mammalian origin to varying degrees, indicating that they all provide the conditions necessary for entry and growth (Wisseman, 1981). For example, studies in our own laboratories (Wisseman, 1981) have shown that *Rickettsia prowazekii* will grow in cells of fibroblastic and epithelial origin in primary, diploid, virus-transformed and malignant states, in human peripheral blood monocytes, lymphocytes, and monocyte-derived macrophages and in human umbilical vein endothelial cells. It readily enters human platelets in suspension (Gaffey, M. and C. L. Wisseman, Jr., unpublished observations). Though the documented observations for *R. rickettsii* are not as extensive, it too seems to exhibit a broad host cell range in vitro (Johnson and Pederson, 1978; Walker *et al.*, 1982). It is unknown if the apparent greater difficulty in demonstrating and establishing growth of *R. tsutsugamushi* in some cell lines is of technical origin, inadequate „adaptation" or some greater "fastidiousness" of the organism. *Coxiella burnetii* seems to infect and grow most readily in cells which display substantial endocytic activity (Baca *et al.*, 1981*a*. *b*; Wisseman, 1981; Baca and Paretsky, 1983).

Entry into host cell (uptake)

Two models, each with specific uses in the laboratory, were studied; (a) a system in which both rickettsiae and cells are in suspension, sedimenting under a force of 1 × g, and (b) a system in which rickettsiae in suspension are sedimenting onto the surface of host cells attached to a surface, as in slide chambers or Petri dishes.

The suspended cell system is by far the more useful, precise, reproducible

and predictable system, in which quantitative relationships can be expressed by simple equations. In this system, isolated host cells sediment under $1 \times g$ force through the medium colliding with rickettsiae whose small sedimentation rate can be ignored. The rate of infection is a function of the collisions between host cells and rickettsiae which in turn is a function of the volume of medium through which the host cell sediments and the rickettsial concentration. It is possible to calculate the theoretical number of collisions between rickettsiae and host cells per unit time, to compare this with the observed rate and calculate the probability that a given collision will result in infection (efficiency of uptake).

When the per cent cells infected is low, the Poisson distribution describes the distribution of rickettsiae quite well. When the per cent infected is high, the normal distribution more closely defines the distribution of rickettsiae. When the ratio of rickettsiae to host cell is high, uptake is relatively independent of host cell concentration and a simple set of equations defines uptake with respect to rickettsial concentration and time in linear fashions. When the ratio of rickettsiae to host cell is low, as may happen with organisms yielding seeds of low titre (e.g., *R. tsutsugamushi*), the influence of host cell concentration becomes more pronounced and, with the addition of this second variable, the system becomes more complex.

This system, properly standardized and calibrated, permits quantitative measurements of the influence of many kinds of variables on the uptake process.

On a more practical plane, with a well-characterized rickettsial seed and a few preliminary measurements, one can prepare on a routine basis cells with any desired degree of infection with a high degree of reproducibility.

The mechanism of *C. burnetii* entry into its host cells has not been studied in detail but appears to be passive, possibly involving attachment to host cell surface followed by endocytosis without passage through any host cell membrane (Baca and Paretsky, 1983). Entry of *Rickettsia* spp. into host cell cytoplasm requires participation by both rickettsia and host cell and involves two sequential temperature sensitive steps: (i) attachment to host cell membrane, possibly through cholesterol-containing receptors, and passage through the host cell membrane accompanied by phospholipase A acitivity (Cohn *et al.*, 1959; Walker and Cain, 1980; Walker and Winkler, 1981; Winkler, 1982; Winkler and Miller, 1981; Wisseman, 1981). The laboratory phenomenon of "lysis from without" in the presence of high concentrations of *Rickettsia* spp. has been observed from time to time over the last 25 years (Wisseman, 1981). Also, in our earlier studies on uptake kinetics described above, we observed that, in the presence of very high *R. prowazekii* concentrations, there was progressive loss of chicken embryo fibroblasts from suspensions without net increase in trypan blue positive cells, suggesting rapid complete lysis when a sufficient number of rickettsiae act on the plasma membrane in a short period of time. Winkler and associates (Winkler and Miller, 1981, 1982) have shown that lysis from without of L929 cells takes place with associated phospholipase A activity when about 50 *R. prowazekii* are brought into contact with the cell membrane by centrifugation.

Intracellular growth

Considerable diversity exists among the different "rickettsiae" with respect to the details of their association with host cells, i.e., their microhabitat. *Rochalimaea quintana*, capable of growth in artificial acellular media assumes a *pericellular* location both in the midgut of its louse vector and in cell culture to receptors on the plasma membrane.

Even among those organisms that are considered *obligate intracellular* parasites, there is considerable diversity of microhabitat. *Coxiella burnetii*, passively entering host cells through endocytosis, remains within a membrane-bound phagolysosome where it replicates through special adaptation to phagolysosomal conditions (see below) (Baca and Paretsky, 1983). *Rickettsia* spp. which pass through the host cell plasma membrane into the cytoplasm, are not surrounded by a host cell membranous structure but are "free", presumably intermingling with host cell organelles and other components (Anderson *et al.*, 1965; Kokorin *et al.*, 1978; Wisseman, 1981), as so beautifully demonstrated in time-lapse cinemicrography by Kokorin at the first International Symposium on Rickettsiae and Rickettsial Diseases in 1967. The significance of fibrils extending between rickettsiae and between rickettsiae and cytoplasmic components in *R. rickettsii*-infected cells remains to be determined (Todd *et al.*, 1983, 1984), as does that of the slime layer (Silverman and Wisseman, 1978; Silverman *et al.*, 1978). *R. prowazekii*, *R. mooseri* and *R. tsutsugamushi* remain largely, if not solely, within the cytoplasm during their growth phase. Though *R. tsutsugamushi* may begin to replicate in a perinuclear microcolony, it may also be found distributed throughout the cytoplasm (Bozeman *et al.*, 1956; Anderson *et al.*, 1965; Urakami *et al.*, 1982*a*). Under light microscopy, all *Rickettsia* spp. may be found from time to time in slender projections from the cell periphery (see also below). *R. canada* and members of the spotted fever group also multiply in the cytoplasm but, in addition in a small proportion of cells, may penetrate through the nuclear membranes into the nucleoplasm and multiply *intranuclearly* (Silverman and Wisseman, 1979; Wisseman, 1981). *R. tsutsugamushi* has been seen within the nucleus (Urakami *et al.*, 1928*b*).

All rickettsiae studied to date appear to replicate primarily by binary fission, although *Coxiella burnetii* also exhibits a "sporogenous-like" cycle which produces minute dense infectious forms (Khavkin *et al.*, 1981; McCaul and Williams, 1981; Weiss, 1982; Baca and Paretsky, 1983). The recent reports of a different kind of replicative cycle for *R. tsutsugamushi* suggesting de novo intracellular assembly from components in the cytoplasm are difficult to interpret but may be the result of technical problems with electron microscopy in uncontrolled cultures of unknown growth phase (Hase, 1983). No counterpart, e.g., an "eclipse" phase, has been recognized by light microscopy.

Microscope examination of stained rickettsia-infected cells in slide culture chambers at intervals after infection permits one to construct growth curves which yield much information, which are amenable to quantitation and which are useful for the testing of the influence of many variables on growth. Different *Rickettsia* species yield different types of growth patterns which

seem to be reproducible and characteristic either of a species and its strains (as with *R. prowazekii* or *R. mooseri*) or of all studied species within a group (as in the spotted fever group).

All strains of *R. prowazekii* studied to date, whether of high egg passage (virulent Breinl, attenuated Madrid E) or of low passage (Burundi, flying squirrel), follow a similar growth pattern (Wisseman and Waddell, 1975; Wisseman *et al.*, 1976*b*; Wisseman, 1981; Wisseman, C. L. Jr., Waddell, A., and Creemer, M. L., to be published). Following entry into host cell cytoplasm, the organisms undergo an intracytoplasmic replication cycle similar to the classical bacterial growth cycle in fluid medium, with *lag*, *exponential growth* and *stationary* phases clearly identifiable and measurable. At 32 °C, the lag phase may be negligible or as long as 5—6 hr, depening upon whether the seed was in an actively growing or stationary phase. The generation time is ~9—11 hr at 32 °C. Morphological changes similar to those exhibited by other gram negative bacilli accompany the different growth phases. The organisms remain within the host cell during the entire 40—48 hr required to fill the cytoplasm and enter the late or stationary phase (constant per cent cells infected), at the end of which time they are released (see below) to infect other cells as reflected by a sharp increase in per cent cells infected.

All strains of *R. mooseri* tested to date (high eg passage Wilmington strain and low passage Ethiopian and Pakistani strains) show growth patterns similar to one another but different from that of *R. prowazekii* strains (Wisseman, C. L. Jr., Waddell, A., and Cremer, M. L., to be published). Although replication takes place in the host cell cytoplasm, the organism are not confined to the host cell. Instead, from the earliest times measurable after infection, these organisms tend to escape from the host cell and infect other cells in the culture, causing a steadily rising per cent of cells infected — a *spreading* type of infection.

R. rickettsii and the other spotted fever group species tested (e.g., *R. conorii*, *R. sibirica*, *R. akari*, *R. australis*, Pakistan isolates) (Wisseman *et al.*, 1976*a*; Wisseman C. L. Jr., and Steiman, I., to be published) also escape from the host cell from the earliest measurable time points and produce a *spreading* type of infection as described above. In addition, in late cultures (3—5 days) compact *intranuclear* masses of rickettsiae are recognizable in a small proportion (~2—5%) of the infected cells.

Although *Coxiella burnetii* is now known to replicate in a host cell phagolysosome (see above), little is yet known about the details.

Escape from host cell

R. prowazekii escapes its host cell at the end of its growth cycle by an unusual mechanism distinct from ordinary cell lysis. Kinetic studies of infected fibroblast cultures show that, during the period of rickettsial release, the cells remain attached to the culture dish, become permeable to trypan blue and release cytoplasmic lactic dehydrogenase, leaving an attache trypsin-sensitive, crystal violet stainable cell residue or "skeleton" (Wisseman, C. L. Jr., Waddel, A., in preparation). Scanning electron micrographs

show cells with large irregular defects in the plasma membrane through which rickettsiae spill out (Silverman *et al.*, 1980).

R. rickettsii and *R. mooseri* escape from their host cells in the early phases of infection without apparent damage to the cells. This was first observed with *R. rickettsii* by Schahter *et al.* (1957) by continuous observation of infected cells by phase contrast microscopy. Scanning electron micrographs of *R. rickettsii* in culture and transmission electron micrographs of *R. tsutsugamushi* in mouse peritoneal mesothelium (Ewing *et al.*, 1978; Wisseman, 1981) show intracellular organisms located in the tip of filiform projections from the host cell surface. With *R. tsutsugamushi* infection of mouse peritoneal mesothelial cells, electron microscopic studies showed that such membrane-covered organisms may be phagocytized by nearby mesothelial cells (Ewing *et al.*, 1978), suggesting intercellular transmission without exposure to extracellular environment. It is unknown if this also occurs in cell cultures. However, in the supernatant fluid of cell cultures infected with *R. rickettsii*, some organisms are found enveloped to varying degrees with host cell membrane which appears to be subsequently discarded, leaving free, uncovered organisms (Silverman *et al.*, 1981). The potential for rickettsiae to spread from cell to cell without contact with the extracellular environment has important implications with respect to avoidance of certain host defense mechanisms — e.g., antibody, phagocytic cells.

Late in the infection cycle of *R. rickettsii* in fibroblast cultures, the organisms are trapped intracellularly by the greatly distended endoplasmic reticulum revealed by transmission electron microscopy (Silverman and Wisseman, 1979 Walker and Cain, 1980). Light microscopy shows that host cells break down and "fragment", releasing organisms and small to large cytoplasmic fragment, containing organisms (Wisseman *et al.*, 1976*a*).

Persistent infections

In contrast to the productive "lytic" cycles described above, cultures of cells persistently infected with *C. burnetii* (Baca *et al.*, 1981*b*; Baca and Paretsky, 1983) and *R. rickettsii* (Todd *et al.*, 1982) have been established. Such cultures offer unique opportunities for study of certain aspects of rickettsia-host cell interaction. Whether or not these in vitro models will provide clues to the nature and mechanism of persistent infections in vivo remains to be determined.

Some considerations of intracellular rickettsial growth

Information about such matters as nutritional requirements of rickettsiae and various external and internal factors that influence intracellular growth is sparse and fragmentary and does not yet yield a coherent pattern. It is becoming increasingly clear, however, that, instead of being leaky organisms, those undamaged rickettsiae studied possess elaborate transport mechanisms as well as complex independent metabolic systems for energy production and the synthesis of macromolecules (Weiss, 1982; Winkler, 1982).

Growth within phagolysosomes

C. burnetii appears to be uniquely adapted to growth within phagolysosomes, even those of macrophages in the presence of lysosomal enzyme and low pH (Hackstadt and Williams, 1981, 1984; Baca and Paretsky, 1983; Baca *et al.*, 1984). Its enzymes show optimal activity at pH 4—5, typical of phagolysosomes, and are essentially inactive in the pH 7—8 range typical of host cell cytoplasm. Whether its nutrients are derived from degradation products of lysosomal contents, as has been suggested, from substances diffusing or transported into the phagolysosome from host cell cytoplasm, or both remains to be determined. Nevertheless, the prospects for cultivation in artificial acellular medium appear to be promising.

Changes induced in host cells by rickettsial infection

Simple infection with 1—3 *R. prowazekii* rapidly renders human fibroblasts in culture susceptible to cytolysis by immune interferon (IFN-γ), even when protein synthesis and growth of the rickettsia have been inhibited with chloramphenicol (Wisseman and Waddell, 1983). The nature of the change induced in the host cell by entry of the organism into the cytoplasm is unknown, but it may involve some alteration in the plasma membrane. L929 cells in cultures persistently infected with *C. burnetii* show quantitative changes in plasma membrane peptides (Baca and Paretsky, 1983).

A very wide range of host cell damage is displayed by various cells infected with different *Rickettsia* spp. At one extreme, cells may show severe damage very rapidly after entry of viable rickettsiae. Rabbit polymorphonuclear leukocytes (PMN) which had phagocytized numerous *R. prowazekii* released lactic dehydrogenase and lost phagocytic capacity (Walker and Winkler, 1981) and guinea pig casein-induced peritoneal PMNs showed glycogen autophagosomes within 30 min after phagocytizing *R. tsutsugamushi* (Rikihise and Ito, 1982; Rikihisa, 1984). A subpopulation of human monocyte-derived macrophages in culture showed microscopic evidence of cell damage and released lactic dehydrogenase into the medium soon after phagocytizing *R. prowazekii* and subsequently failed to support rickettsial growth (Meyer, W. A., and Wisseman, C. L., Jr., to be published). In each of these instances, the cell was a "professional phagocyte" and the organisms gained entry by phagocytosis and subsequently escaped from the phagocytic vacuole into the cytoplasm. At the opposite extreme are the persistently infected cultures in which both rickettsia and host cells multiply for many generations (Baca *et al.*, 1981*b*; Todd *et al.*, 1982; Baca and Paretsky, 1983).

In the usual productive infection, however, once *Rickettsia* spp. enter the cytoplasm, there seems to be a balance between rickettsial replication and rickettsia-induced host cell damage which permits a substantial increase in organisms before the host cells are destroyed. In the early phases of infection, host cells may continue to replicate, increase replication or cease growth (Bozeman *et al.*, 1956; Hopps *et al.*, 1959*a*, *b*; Kokorin *et al.*, 1978; Wisseman, C. L. Jr., and Waddell, A, in preparation).

Transmission electron microscopy of ultrathin sections of *R. prowazekii*-

-infected chicken embryo fibroblasts showed very little evidence of damage to host cell structures and organelles, even late in the infection when the cytoplasm was packed with organisms (Silverman *et al.*, 1980). On the other hand, *R. rickettsii*-infected cells showed mainly large distention of the endoplasmic reticulum, loss of Golgi apparatus and later loss of integrity of the plasma membrane (Silverman and Wisseman, 1979; Walker and Cain, 1980). In both systems, mitochondria showed relatively minor changes, until late in the infection cycle. Cytochemical studies by Kokorin *et al.* (1978) of cells infected with various rickettsiae showed that, while some lysosomal enzymes may show a rise and subsequent fall in activity, mitochondrial enzymes were minimally affected.

Differences among *Rickettsia* spp. in apparent action on host cell membranes is provocative (Wisseman, 1981, 1983). All show a capacity to pass through the plasma membrane from the external surface into the host cell cytoplasm. *R. canada* and spotted fever group rickettsiae can also pass through the nuclear membranes into the nucleus. *R. prowazekii* does not escape from undamaged host cells, but rupture of penicillin-induced spheroplasts may be accompanied by host cell lysis (Wisseman *et al.*, 1982). Although endoplasmic reticulum of *R. rickettsii*-infected cells shows evidence of functional damage (? loss of water regulation), the rickettsiae do not pass through this membrane to enter the cisternae. Electron microscope studies of *R. rickettsii* plaques has yielded similar information about the sequence and nature of host cell damage (Walker and Cain, 1980).

The selectivity of action on different host cell membranes may have survival value for the rickettsiae. An early action on lysosomal membranes which would release lysosomal enzymes into the cytoplasm and possibly cause host cell "suicide" would not be favorable to rickettsial survival. In human monocyte-derived macrophages, *R. prowazekii* has been observed repeatedly in contact with thorium dioxide labeled lysosomes without evidence of loss of integrity of the lysosomal membrane (Meyer, W. A., and Wisseman, C. L., Jr., in preparation). However, it is possible that lysosomes may break down late in the infection and may contribute to host cell disruption. Protease and phospholipase A action have been suggested as contributing to host cell injury by *R. rickettsii* (Walker *et al.*, 1983, 1984). Enhanced growth of *R. typhi* observed in cortisone-treated L cells was attributed to lysosomal stabilization (Woodman *et al.*, 1979).

Growth "free" in host cell cytoplasm

Some properties of studied *Rickettsia* spp. are consistent with adaptation to intracytoplasmic growth —e.g., optimal organismal stability and enzyme activity at $\sim$ pH 7.4, sparing of lysosomal and mitochondrial membranes.

Host cell contribution to growth of *Rickettsia* spp. still is known better by what is *not* required than by what is required. They do not offer special protection from osmotic forces (Myers *et al.*, 1967). Host cell nucleus does not appear to be essential. Rickettsial growth has been demonstrated in cells (a) arrested in the metaphase by colchicine (Bozeman *et al.*, 1956), (b) whose

DNA has been cross-linked by mitomycin C (Wisseman, 1981) or (c) whose nucleus has been removed (Stork and Wisseman, 1976). Inhibition of host cell protein synthesis by cycloheximide or emetine does not halt rickettsial growth (Weiss *et al.*, 1972, 1973; Oaky *et al.*, 1981).

The interesting phenomenon of "reactivation" of *R. rickettsii* in starved ticks by feeding and elevation of temperature is correlated with the re--acquisition (synthesis) of the microcapsular and slime layers (Hayes and Burgdorfer, 1982).

In the case of factors present in the medium, it is often difficult to distinguish between (a) a direct action on the intracellular rickettsiae and (b) an indirect action mediated through the host cell. Thus, elimination of serum from ordinary cell culture media inhibited both host cell and rickettsial growth (Hopps *et al.*, 1959*a*). *R. prowazekii*, *R. mooseri* and *R. ricketsii*, but not *R. tsutsugamushi* require a CO_2-enriched culture atmosphere (Kopmans--Gargantiel and Wisseman, 1981). Radiolabeled amino acids in the medium are incorporated into rickettsiae even when host cell protein synthesis is inhibited with cycloheximide or emetine (Weiss *et al.*, 1972, 1973; Oaks *et al.*, 1981; Walker and Winkler, 1981). Nucleotides and galactose in the medium have been incorporated into intracellular rickettsiae (Weiss, 1982). Antibiotics in the culture medium inhibit intracellular rickettsial growth (Wisseman *et al.*, 1974). Much more work along these lines, coupled with studies of metabolic and transport activities of cell-free rickettsiae (Winkler, 1982), needs to be done.

A special instance of an external substance influencing intracellular rickettsial viability through the medium of the host cell, of potential importance in cell mediated immune control of *R. prowazekii* infection in the intact mammalian host, has recently been discovered. Immune interferon (IFN-γ), in addition to the cytolytic effect on *R. prowazekii*-infected cells (above), causes protein synthesis-dependent change(s) in the host cell which results in the intracytoplasmic death of *R. prowazekii* in endothelial cells, macrophages and fibroblasts in culture (Turco and Winkler, 1983; Wisseman and Waddell, 1983). The mechanism is unknown, but it may be mediated through one or more of those proteins induced by IFN-γ and not by IFN-α or IFN-β (Wisseman, C. L. Jr., Waddell, A., and Ordonez, S., in preparation). Lymphokines also restrict growth of *R. tsutsugamushi* in mouse macrophages (Nacy *et al.*, 1981).

Observations on rickettsial infection of endothelial cells and macrophages in cell culture

Because endothelial cells and possibly macrophages are specially involved in rickettsial infections in the intact mammal, some brief special attention to in vitro studies is warranted, though information is still severely limited.

R. prowazekii infects human umbilical vein endothelial cells in culture and undergoes a growth cycle similar to that described above for fibroblasts (Wisseman and Waddell, 1983). An unexplained anecdotal observation from initial exploratory experiments with bovine aortic and human umbilical vein

endothelial cell cultures, in which technical problems cannot be excluded, was that large numbers of *R. prowazekii* were initially associated (attached and/or intracellular) with the endothelial cells during the infection period but, that after 24 hr incubation, very few organisms could be found (Wisseman, C. L. Jr. and Waddell, A., unpublished data). More recently, a detailed study of the adherence and entrance of the attenuated Madrid E strain of *R. prowazekii* with human umbilical vein endothelial cells indicated that the signal for entry probably involved creation of a calcium gradient and that there was a limit to the number of attached rickettsiae which gained entry into the cells (Walker, 1984). Treatment of human umbilical vein endothelial cells in culture with human IFN-γ-containing lymphokines caused death of the intracellular *R. prowazekii* (Wisseman and Waddell, 1983). *R. ricketsii* undergoes a spreading type of infection cycle in cultured human umbilical vein cultures just as it does in chicken embryo fibroblasts, induces plaque formation in monolayers of human umbilical vein endothelial cells under agarose, and causes cell damage similar to that described for chicken embryo fibroblasts (Walker *et al.*, 1982; Silverman, 1884; Silverman and Bond, 1984, Walker, 1984; Silverman, D. J., this symposium). Studies on the interaction of *R. rickettsii*-damaged endothelial cells with platelets as a possible factor in the observed disturbances in intravascular coagulation are in progress (Silverman, D. J., personal communication).

The literature on the interaction between rickettsiae and cells of the monocyte-macrophage series is expanding. A comprehensive consideration is beyond the scope of this review and only very selected observations of the interaction between typhus rickettsiae and *C. burnetii* and macrophages are presented. Both the virulent Breinl strain of *R. prowazekii* and the virulent Wilmington strain of *R. mooseri* are phagocytized by human monocyte--derived macrophages in culture, but both species appear to inhibit lysosomal fusion and escape from the phagosome through a created defect in the phagosomal membrane and enter into the macrophage cytoplasm where they undergo a normal growth cycle (Andrese and Wisseman, 1971; Beaman and Wisseman, 1976; Gambrill and Wisseman, 1973*a*; Meyer, W. A., and Wisseman, C. L. Jr., to be published). Pretreatment of the rickettsiae with typhus convalescent serum or monoclonal antibodies to the 138 kD surface protein results in enhanced phagocytosis, restriction of the organism to the phagosome, lysosome-phagosome fusion and rapid destruction of the rickettsiae in the phagolysosome (Andrese and Wisseman, 1971; Beaman and Wisseman, 1976; Gambrill and Wisseman, 1973*a*, *b*; Oaks, E. V., and Wisseman, C. L. Jr., to be published; Meyer, W. A., and Wisseman, C. L., Jr., to be published). Treatment with IFN-γ-containing lymphokines causes intracytoplasmic destruction of *R. prowazekii* (Wisseman and Waddell, 1983).

The attenuated Madrid E strain of *R. prowazekii* grows as well as the Breinl strain in chicken embryo cells (Wisseman and Waddell, 1975) but, in contrast to the Breinl strain, is destroyed by human peripheral blood monocyte--derived macrophages in culture (Gambrill and Wisseman, 1973*a*). The virulent and attenuated strains could be differentiated by capacity to grow in

a series of macrophage-like cell lines (Turco and Winkler, 1982). There also appeared to be differences in the capacity of the virulent and attenuated strain to grow in the cytoplasm of infected L-929 cells which were subsequently fused with a macrophage-like cell line. Gudima (1979, 1982), using the criterion of capacity for serial passage in culture, also showed differences between the virulent and attenuated strains in a series of non-macrophage cell lines.

C. burnetii grows within macrophage phagolysosomes. The growth of Phase I and Phase II *C. burnetii* has been compared in a series of macrophage-like cell lines (Baca *et al.*, 1981*a*, *b*; Baca and Paretsky, 1983) which show different degrees of lysosomal fusion with phagosome. Reports on the effect of pretreatment with antibody on the intraphagolysosomal fate of *C. burnetii* have been contradictory, varying from inhibition of growth to no effect (Baca and Paretsky, 1983). Cell mediated immune mechanisms, nature not yet elucidated, also restrict *C. burnetii* replication in macrophages (Baca and Paretsky, 1983).

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