The Pathogenesis of Systemic Chlamydial Infections: Theoretical Considerations of Host Cell Energy Depletion and Its Metabolic Consequences

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Introduction
The recent observation that *Chlamydia pneumoniae* can infect human endothelial cells in vivo has led to speculation that such chronic infections might play a role in the pathogenesis of atherosclerosis. Regardless of any role in atherosclerosis, the fact that this pathogen can chronically infect endothelial cells, monocytes, smooth muscle cells, and perhaps other cells raises a number of theoretical considerations related to chlamydial energy requirements and their subsequent effects on the host cell. The following discussion addresses host-cell energy depletion and its possible metabolic consequences as these metabolic consequences could be important in the pathogenesis of systemic chlamydial infections.

Chlamydial Requirements from Eukaryotic Host Cells
Chlamydiae are prokaryocytes that develop in eukaryotic cells and utilize part of the host cell metabolism. Becker and Asher have noted that the transition of elementary bodies (EBs) to reticulate bodies (RBs) for *C. trachomatis* requires the presence of functioning mitochondria in the infected cell as well as the production by the host cell of nucleoside triphosphates which are needed for chlamydial biosynthesis of nucleic acids. Chlamydiae are known to possess fragments of the glycolytic, pentose phosphate, and citric acid pathways and appear to be capable of converting glucose-6-phosphate (but not glucose) to pyruvate and pentose. However, chlamydiae seem to lack enzymes needed for the net generation of adenosine triphosphate (ATP). Thus, chlamydial development is dependent on active mitochondrial and nuclear function of the host cell. For this reason, chlamydiae are considered obligatory intracellular parasites.

Chlamydia and Host Cell Mitochondria
The requirement of an exogenous source of ATP and the presence of a specific ATP transport system in chlamydiae have provided supporting evidence for the energy parasite concept. Hatch and colleagues have demonstrated that this ATP transport system is an ATP-adenosine diphosphate (ADP) exchange mechanism similar to that found in mitochondria by Duee and Vignais. Moreover, electron microscopic studies have shown that replicating chlamydiae are always found in close proximity to mitochondria. Therefore, it has been suggested that chlamydiae behave in the reverse manner of mitochondria in that mitochondria import ADP from the host cell cytoplasm and export ATP, while chlamydiae import ATP and export ADT.


definition of the host cell's energy needs at the net expense of depriving host cell biosynthetic pathways. This obligatory depletion in host cell ATP due to intracellular chlamydial infection has been demonstrated by Gill and Stewart. These investigators used an in vitro cell culture model in which they demonstrated that a decline in host cell ATP levels was accompanied by a concomitant increase in ADP levels. Two additional studies, one by Horoschak and Moulder and another by Bose and Liebhaber, have demonstrated that a high multiplicity of infection of host cells with chlamydiae quickly brings host cell division to a halt, whereas lower multiplicities slow, but do not immediately stop, the division of host cells.

Chlamydia and Host Cell Energy
Chlamydial dependence on host cell energy must necessarily deplete the host cell’s existing energy output at the net expense of depriving host cell biosynthetic pathways. This obligatory depletion in host cell ATP due to intracellular chlamydial infection has been demonstrated by Gill and Stewart. These investigators used an in vitro cell culture model in which they demonstrated that a decline in host cell ATP levels was accompanied by a concomitant increase in ADP levels. Two additional studies, one by Horoschak and Moulder and another by Bose and Liebhaber, have demonstrated that a high multiplicity of infection of host cells with chlamydiae quickly brings host cell division to a halt, whereas lower multiplicities slow, but do not immediately stop, the division of host cells.

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phenomenon are likely to be energy related. The effect of chlamydia-related energy deprivation on host cell biosynthetic pathways has been noted in vivo as well. For example, Kouil and colleagues have shown in experimental mastitis in goats that chlamydial infection results in dysfunction of acinar cells of udder, teat sinus epithelium, hepatocytes, and renal tubules up to 34 days after experimental infection.

Mitochondrial Generation of Energy in Eucaryotic Cells
As chlamydiae are energy parasites of eucaryotic cells, it is useful to review the mitochondrial function of these host cells as cellular energy is provided by these organelles. The mechanism for the mitochondrial generation of energy is a function of the cellular ultrastructure. Each intracellular mitochondrion has two distinct membranes, an inner and an outer membrane, that define functional spaces. An intermembrane space is defined by the outer and inner mitochondrial membranes. The inner mitochondrial membrane defines an interior space, or compartment, known as the mitochondrial matrix. It is the mitochondrial matrix which contains the specialized proteins of the electron transport chain (respiratory chain) and the enzymes of the citric acid cycle (Krebs cycle) responsible for the generation of ATP. The inner membrane has a very large surface area due to its characteristic infolds, known as cristae. This inner membrane contains the electron transport carriers (primarily cytochromes). It also contains a high concentration of cardiolipin, a phospholipid believed to decrease the permeability of this bilayer to small ions. The outer mitochondrial space has direct access with the cytosol via the outer membrane which contains major integral proteins called porins. Porins can form channels within the outer membrane through which molecules that are less than 10,000 MW (<10 KDa) can freely pass from the cytosol.

The production of ATP within the mitochondria is powered by a mechanism called chemiosmotic coupling (Figure 1). This process begins with the conversion of glucose to pyruvate in the cytoplasm by the glycolytic pathway (Embden-Meyerhoff pathway). The net reaction produces two reduced nicotinamide adenine dinucleotide (NADH) molecules that serve as the principal electron donors for the reduction of oxygen. The two NADH molecules as well as pyruvate enter the mitochondrial matrix. Pyruvate is converted to acetyl-coenzyme A which is the key substrate for the citric acid cycle. The metabolism of pyruvate in the mitochondria is assisted by two cofactors, biotin and lipoic acid. Fatty acids also enter the mitochondrial matrix where they are oxidized to acetyl-coenzyme A. Both pyruvate and fatty acids thus fuel the citric acid cycle. During the citric acid cycle, acetyl-coenzyme A is oxidized to two molecules of CO2 which is released from the cell; the electrons released by this reaction are transferred to NAD and flavin adenine dinucleotide (FAD). The three NADH and FADH2 molecules created by the citric acid cycle as well as the NADH generated by glycolysis are oxidized — a process which transfers electron pairs to acceptor molecules on the inner mitochondrial membrane, eventually leading to the reduction of oxygen and the formation of water.

The oxidation of NADH or FADH2 releases a hydride ion (H+), which is quickly converted to a proton (H+) and two high-energy electrons (2 e-). These two high-energy electrons reduce molecular oxygen to water during mitochondrial respiration. This transfer of electrons from NADH or FADH2 to oxygen is catalyzed by a series of electron carriers that are associated with multiprotein complexes. These complexes include NADH dehydrogenase, cytochrome oxidase, and ATP synthetase (ATPase). The high-energy electrons are transferred to ubiquinone by a flavin and several iron-sulfur prosthetic groups attached to the multiprotein complexes. The hydrophobic ubiquinone then moves laterally in the inner membrane and transfers the electrons to the b-c
multiprotein complex. Cytochrome c is another soluble electron carrier that, in turn, transfers electrons between multiprotein complexes III and IV.

As the high-energy electron pair is transferred to each of these three multiprotein complexes, the protons produced pass freely from the mitochondrial matrix to the intermembrane space via channels in complexes I, III, and IV. Thus, the transfer of electrons from NADH down the electron transport chain causes protons to be pumped out of the mitochondrial matrix and into the intermembrane space. These protons then reenter the matrix through a specific channel in complex V. This proton gradient across the inner membrane results in the proton motive force which drives ATP synthesis.

ATP synthesis is carried out in sub-mitochondrial elementary particles which use the electron pair from NADH of FADH₂ to reduce oxygen and convert ADP plus phosphate to ATP. ATP synthetase is reversible, and without the large proton motive force, it could theoretically hydrolyze ATP and use the free energy derived from this hydrolysis to move protons out of the mitochondrial matrix. Because of the large proton motive force, protons move down their electrochemical gradient through ATP synthetase; this proton movement drives ATP synthesis.

Inhibitors of the passage of high-energy electrons to O₂ (e.g., cyanide ion) block ATP synthesis. The converse is also true: inhibition of ATP synthetase blocks electron transfer in the mitochondria. In general, electron transfer and ATP synthetase are obligatorily coupled; neither reaction occurs without the other. However, under certain conditions, mitochondrial oxidation can be uncoupled from phosphorylation. For example, certain chemicals (e.g., 2-4 dinitrophenol) and ionophores (e.g., oligomycin) can uncouple this mitochondrial reaction.

Mechanisms for Chlamydia to Derive Energy from the Host Cell
The transformation of chlamydial elementary bodies (EBs) to reticulate bodies (RBs) is known to initially involve the activation of intrinsic chlamydial ATPase that are contained within the EBs. This ATPase is oligomycin sensitive and magnesium ion dependent and, by definition, is considered an F-type ATPase. This chlamydial F-type ATPase shares both sequential and structural homology with mitochondrial ATP synthetase and, like mitochondrial ATP synthetase, catalyzes the condensation of ADP and inorganic phosphorus (P₂O₅ to form ATP.

Chlamydial ATPase in essence may be competing with host cell mitochondrial ATPase. This, of course, would reduce the ATP produced by the mitochondria. A net reduction of ATP in the host cell mitochondria would result in an concomitant lowering of the electron transfer in the host cell mitochondria because electron transfer and ATP synthesis are obligatorily coupled; neither reaction occurs without the other. The establishment of a large electrochemical proton gradient across the inner mitochondrial membrane by chlamydial infection could halt normal electron transport and might even cause a reverse electron flow in some sections of the host cell respiratory chain. The reduction of electron transfer in the host cell mitochondria, in turn, could lower the translocation and reduction of extramitochondrial ferric iron to intramitochondrial ferrous iron. If this occurred, the energy depletion would interfere with the biosynthesis of heme.

The Biosynthesis of Heme
The biosynthesis of heme is an energy-dependent process which is adversely affected by depletion of host cell energy. Gajdos has demonstrated that the metabolic consequence of the interruption of ATP biosynthesis is a secondary porphyria. A review of the biosynthesis of heme is warranted (Figure 2). Heme synthesis is a series of irreversible biochemical reactions, some of which occur in the cell mitochondria and some in the cytoplasm. The intramitochondrial reactions are mainly oxidation-reduction while those in the cytosol are condensation and decahydroxilation. Porphyrinogens, porphyrins, and porphyrin are all related to heme synthesis. The biosynthesis of heme occurs in all human cells and involves a relatively small number of starting materials which are condensed to form porphyrinogens; the porphyrins are formed from the porphyrinogens by non-enzymatic oxidation. As porphyrinogens progress through the heme biosynthesis pathway, the numbers of carboxyl side groups on the corresponding porphyrins decreases, as does the water solubility of the compounds.

Heme is a Fe²⁺ complex in which the ferrous ion is held within the organic ligand, tetrapyrrolic macrocycle. The heme-containing tetrapyrrolic macrocyclic pigments are known as porphyrinogens and play a major role in cellular biochemistry. A number of critical cellular functions such as electron transport, reduction of oxygen, and hydroxy-
lation are mediated by a family of heme-based cytochromes including catalase, peroxidase, and superoxide dismutase. Moreover, the oxygen-carrying properties of hemoglobin and myoglobin are based on heme. Many cellular enzymes such as cytochrome P-450 and tryptophan pyrrolase contain heme.

The porphyrias are consequences of any impairment of the formation of porphyrinogens or in their transformation to heme. Porphyrins are formed from porphyrinogens by non-enzymatic oxidation. Each of the various genetic porphyrias is linked to a deficiency in the heme biosynthesis pathway. As a consequence of the enzyme defects, there is increased activity of the initial and rate-controlling enzyme of this biosynthetic pathway which results in overproduction and increased excretion of porphyrin precursors and porphyrinogens.

The porphyrias are often considered pharmacogenetic disorders as many pharmacological agents including a number of anticonvulsants such as carbamazepine, phenytoin, phenobarbitone, and primidone are potent inducers of heme biosynthesis and consequently can precipitate biochemical and clinical exacerbations in patients who have the underlying genetic defect.

When porphyrinogens accumulate secondary to enzymatic defects in the heme biosynthesis pathway, they are oxidized to photosensitizing porphyrins. Porphyrins are classified as photodynamic agents because they generally require oxygen to exert their damaging biologic effects. Porphyrins may be converted from ground state to excited state molecules after absorption of radiation. Excited state porphyrins transfer energy to oxygen molecules and produce reactive oxygen species such as singlet oxygen, superoxide anion, superoxide radical, hydroxyl radical, and hydrogen peroxide. Reactive oxygen species have been noted to disrupt membrane lipids, cytochrome P-450, and DNA structure. If these reactive oxygen species are released into the extracellular space, as seen in acute porphyria, autooxidation of surrounding tissue may result. Two cytoplasmic enzymes, superoxide dismutase and catalase, protect cellular contents against reactive oxygen species by destroying O₂⁻ and H₂O₂. In the extracellular milieu, ceruloplasmin, a copper-containing plasma protein, mimics the dismutase activity of superoxide dismutase and protects the extracellular milieu from autooxidation. Thus, the accumulation of porphyrinogens/porphyrins in human tissues and body fluids produces a condition of chronic system overload of oxidative stress with long term effects particularly noted for neural, hepatic, and renal tissue.

The first step of heme biosynthesis is the rate-limiting step and begins in the mitochondria where delta-aminolevulinic acid (δ-ALA) is condensed from glycine and succinyl coenzyme A by the mitochondrial enzyme δ-ALA synthase in the presence of pyridoxal 5'-phosphate. δ-ALA diffuses into the cell cytoplasm after its formation where two δ-ALA molecules are then condensed by the enzyme δ-ALA dehydratase to form the monopyrrole porphobilinogen (PBG). This enzyme, δ-ALA dehydratase, is inhibited by lead and heme. The next step of heme biosynthesis involves the enzyme PBG deaminase which catalyzes the synthesis of the linear molecule of tetrpyrrole hydroxymethylbilane from four molecules of PBG. This reaction is critical as its product, hydroxymethylbilane, can be converted to the asymmetric uroporphyrinogen-III by the action of uroporphyrinogen-III cosynthase, or it can undergo spontaneous non-enzymatic conversion to the symmetric uroporphyrinogen-I. The isometric porphyrinogens formed from uroporphyrinogen-I are metabolic products with no known physiologic function. These also undergo non-enzymatic oxidation to porphyrins.

The enzyme PBG deaminase may have a partial deficiency which results in acute intermittent porphyria which is inherited as a Mendelian autosomal dominant trait. Because of the partial enzyme block in the third step of the pathway of heme biosynthesis, there is increased activity of the initial and rate-controlling enzyme δ-ALA synthase which is under negative feedback control by heme. During clinical attacks and sometimes also during remission, there is overproduction of the porphyrin precursors δ-ALA and PBG which are formed prior to the enzyme defect then are excreted in excess in the urine.

The enzyme uroporphyrinogen-III cosynthase takes the linear molecule of tetrpyrrole hydroxymethylbilane and closes the porphyrin ring. In the absence of the uroporphyrinogen-III cosynthase enzyme, hydroxymethylbilane spontaneously cyclizes to form the isomer of uroporphyrinogen. The enzymatic defect in congenital erythropoietic porphyria is found in the uroporphyrinogen-III cosynthase enzyme. Uroporphyrinogen-III is converted to 7, 6, 5-carboxyl porphyrinogen-III by uroporphyrinogen decarboxylase. Both hepatopaerythropoietic porphyria and porphyria cutanea tarda have a defect in this enzyme.

A critical step in the biosynthesis of heme is the later mitochondrial stages in which the last three enzymes of heme synthesis, coproporphyrinogen oxidase, protoporphyrinogen oxidase, and ferrochelatase drive the concurrent translocation and reduction of extra-mitochondrial ferric iron to ferrous in the mitochondrion (Figure 3). At the same time, coproporphyrinogen-III must also enter the mitochondrion in order to be oxidized to protoporphyrinogen into which the ferrous ion is inserted to form heme. Coproporphyrinogen oxidase converts coproporphyrinogen-III to protoporphyrinogen. Hereditary coproporphyrina is an autosomal dominant disorder in which the genetic defect is a partial deficiency of coproporphyrinogen oxidase. Protoporphyrinogen in turn is oxidized to protoporphyrin by the enzyme protoporphyrinogen oxidase. These oxidative steps take place in the mitochondrial matrix membrane as coproporphyrinogen-III moves from the cytosol back into the matrix. The final step of heme biosynthesis, like the initial, occurs in the mitochondrial matrix where protoporphyrin is chelated with ferrous iron to form heme. The enzyme ferrochelatase catalyzes this last reaction.
in the extracellular milieu. Within the mitochondrial matrix, the final steps in the biosynthesis of heme are halted. Because the accumulation of heme within the mitochondrial matrix normally exerts a negative feedback on heme biosynthesis, the reduction of heme caused by the inability of coproporphyrinogen to return to the mitochondrial matrix results in the increased production of heme precursors such as δ-ALA and PBG, the first and second products in heme biosynthesis. Thus, porphyrin precursors such as δ-ALA and PBG begin to accumulate in the mitochondrial matrix, then in the cytosol, and then in the extracellular milieu.

Depletion of host cell energy by the intracellular infection with Chlamydia species might cause additional energy-related complications. As fewer electrons are available to move through the electron transport chain of the host cell mitochondrial matrix membrane, the citric acid cycle produces more succinyl-CoA which, in turn, promotes increased synthesis of δ-ALA. The net result is an increased amount of heme precursors and hence porphyrins. The presence of porphyrins in the mitochondrial matrix may damage the cell as these molecules are unstable and form free radicals. The high energy electrons generated by these free radicals could be "captured" by ubiquinone and cytochrome c which are present in the mitochondrial matrix membrane. This, of course, would effectively uncouple electron transport from ATP synthesis and "short circuit" the proton-motive force: ATP synthesis would then be reduced. Less ATP, in turn, means increased porphyrins, and a destructive cycle is begun.

The clinical result of the intracellular and extracellular accumulation of porphyrins, if extensive, would be an tissue/organ specific secondary porphyria which might produce the classical manifestations of porphyria, including neuropsychiatric symptoms and signs. As the chlamydial-infected host cells lyse, the hemolytic effect on the normal life cycle of Chlamydia, the intracellular porphyrins are released and result in porphyria. Moreover, if chlamydiae were to infect hepatic cells, the use of any pharmacologic agents that are metabolized in the liver will increase the need for cytochrome P-450 which is a heme-based enzyme. Hence, the biosynthesis of heme in the liver is increased. If hepatic cells were infected with Chlamydia species, the decreased energy in the host cell would not allow heme biosynthesis to go to completion, and porphyrins in the liver/entero-hepatic circulation would increase. It also might be predicted that any host cell infected with Chlamydia species could have an increased amount of intracellular porphyrins that would be released when antimicrobial agents kill the microorganism. The theoretical metabolic disorder described clearly would be of paramount importance in dealing with chronic systemic chlamydial infections as might be seen with intravascular infections caused by Chlamydia pneumoniae.

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