preventive and/or specific adjunctive therapy.

The first of these concomitant disorders is a porphyria which is a direct result of the chlamydial infection of host cells. This form of porphyria is a secondary porphyria as it is not the result of a genetic deficiency of the enzymes involved in the biosynthesis of heme. Based upon the discovery of this secondary form of porphyria, a unique approach for the diagnosis and treatment of obligatory and secondary disorders caused by Chlamydia infections has been developed. The adjunctive therapy described herein can be used in combination with the appropriate antimicrobial therapy required for eradication of the pathogen. This adjunctive therapy for secondary porphyria is particularly important for long-term antimicrobial therapy of chronic/systemic infections as such therapy often evokes symptoms of secondary porphyria.

The discussion below outlines the believed mechanism by which Chlamydiae induce these secondary metabolic disorders. The phrase "chlamydial-induced porphyria" is defined herein as an obligatory and secondary metabolic disorder which is the direct result of a chlamydial infection and which may find clinically relevant phenotypic expression requiring interventional therapy.

Chlamydiae are prokaryocytes that develop in eukaryotic cells and utilize part of the host cell metabolism (Becker, Y., Microbiological Reviews, 42:247-306 (1978); McClairty, G., Microbiology, 2:157-164(1994)). The transition of elementary bodies (EBs) to reticulate bodies (RBs) for Chlamydia species 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 (Becker, Y., Microbiological Reviews, 42:247-306 (1978); McClairty, G., Microbiology, 2:157-164(1994); Ormsbee, R. A. and Weiss, E., Science, 2:1077 (1963); Weiss, E., Jour. of Bacteriology, 90:243-253 (1965); Weiss, E. and Kiesow, L. A., Bacteriology Proceedings, 85 (1966); Weiss, E. and Wilson, N. N., Jour. of Bacteriology, 97:719 (1969); Hatch et al., Jour. of Bacteriology, 150:662-670 (1985)).

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 (Ormsbee, R. A. and Weiss, E., Science, 2:1077 (1963); Weiss, E. and Kiesow, L. A., Bacteriology Proceedings, 85 (1966)). However, Chlamydiae seem to lack enzymes needed for the net generation of adenosine triphosphate (ATP) (Weiss, E., Jour. of Bacteriology, 90:243-253 (1965)). Thus, chlamydial development is dependent on active mitochondrial and nuclear function of the host cell. For this reason, Chlamydiae are considered obligatory intracellular parasites (McClairty, G., Microbiology, 2:157-164(1994)). 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.

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 et al., Jour. of Bacteriology, 150:662-670 (1985)). This ATP transport system is an ATP-adenosine diphosphate (ADP) exchange mechanism (Peeling et al., Infect. and Immun., 57:3334-3344 (1989)) similar to that found in mitochondria (Penefsky, H. S. and Cross, R. L., Adv. Enzym. and Rel. Areas in Molec. Bio., 64:173-214 (1991)). 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 ADP (Becker, Y., Microbiological Reviews, 42:247-306 (1978)).

The production of ATP within the mitochondria is powered by a mechanism called chemiosmotic coupling (Kalkcar, H. M., Annu. Review of Biochem., 60:1-37 (1991); Lehninger, A. L., The Mitochondrion: Molecular Basis of Structure and Function, The Benjamin Company, Incorporated, New York; Slater, E. C., Europ. Journal of Biochem., 166:489-504 (1987); Babcock, G. T. and Wickström, M., Nature, 365:301-309 (1992); Senior, A. E., Physiology Review, 68:177-231 (1988); Pedersen, P. I. and Carafoli, E., Trends in Biochem. Sci., 12:145-150 (1987); Pedersen, P. I. and Carafoli, E., Trends in Biochem. Sci., 12:145-150 (1987)). The citric acid cycle drives oxidation of NADH or FADH2, which, in turn, releases a hydride ion (H-), which is quickly converted to a proton (H+)and two high-energy electrons (2 e-). As the high-energy electron pair is transferred to each of these three multiprotein complexes, the protons produced pass freely from the mitochondria 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.

Chlamydial ATPase in essence is competing for protons with host cell mitochondrial ATPase. This, of course, reduces the ATP produced by the mitochondria. A net reduction of ATP in the host cell mitochondria results in a 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 halts normal electron transport and can 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, lowers the translocation and reduction of extramatrix mitochondrial ferric iron to intramatrix ferrous iron. This energy depletion, in turn, interferes with the biosynthesis of heme.

A. Biosynthesis of Heme

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 hydroxylation 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 tryprophan pyrolase contain heme.


Porphyrinogens, porphyrins and porphyria are all related to heme synthesis. The biosynthesis of heme occurs in all human cells and involves a relatively small number of starting materials that 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.

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 an enzyme 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 biosynthesis pathway that results in overproduction and increased excretion of porphyrinogen precursors and porphyrinogens. The steps of heme biosynthesis are laid out in Table 7.

**TABLE 7**

Simplified outline of enzymes and precursors in the Biosynthesis of Heme

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Other precursor</th>
<th>Inhibitor</th>
<th>Result¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Δ-ALA synthase</td>
<td>pyridoxal 5’-phosphate</td>
<td>heme</td>
<td>glycine and succinyl coenzyme A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>heme</td>
<td>delta-aminolevulinic acid (Δ-ALA)</td>
</tr>
</tbody>
</table>
Δ-ALA dehydratase* lead and heme porphobilinogen (PBG)
PBG deaminase* tetrapyrrole hydoxymethylbilane
uroorphyrinogen-III cosynthase* uroporphyrinogen-III
uroorphyrinogen decarboxylase* 7,6,5-carboxyl porphyrinogen-III
coproporphyrinogen oxidase coproporphyrinogen-III
protoporphyrinogen oxidase protoporphyrinogen
ferrochelatase ferrous ion protoporphyrin

In the absence of this step, the symmetric uroporphyrinogen-I is formed

Becomes precursor of the next step

*Present in circulating red cells

When porphyrinogens accumulate due to enzymatic defects in the heme biosynthesis pathway, they are oxidized to photosensitizing porphyrins. Porphyrins are classified as photodynamic agents because they generally require superoxide/oxygen/electrons 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, super oxide 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. 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.

B. Chlamydia and Secondary Porphyria

As mentioned, ferric/ferrous translocation is a critical step in the biosynthesis of heme as it catalyses the oxidative entry of coproporphyrinogen into the mitochondria matrix as protoporphyrin; Chlamydia interfere with this step by reducing electron transfer in the host cell. When coproporphyrinogen is unable to return to the mitochondrial matrix, it accumulates first in the cytosol and then 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 causes 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 which become porphyrins. The presence of porphyrins in the mitochondrial matrix damages the cell as these molecules are unstable and form free radicals. The high energy electrons generated by these free radicals is "captured" by ubiquinone and cytochrome c which are present in the mitochondrial matrix membrane. This, of course, effectively uncouples electron transport from ATP synthesis and "short circuits" the proton-motive force: ATP synthesis is then 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, is a tissue/organ specific porphyria which produces many of the classical manifestations of hereditary porphyria. As the chlamydial-infected host cells lyse, as happens in the normal life cycle of Chlamydia, the intracellular porphyrins are released and result in a secondary porphyria. Moreover, when the chlamydial infection involves hepatic cells, the use of any pharmacologic agents that are metabolized by cytochrome P-450 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 becomes increased. When hepatic cells are infected with Chlamydia species, the decreased energy in the host cell does not allow heme biosynthesis to go to completion and porphyrins in the liver/entero-hepatic circulation are increased. It also has been noted that any host cell infected with Chlamydia species has an increased amount of intracellular porphyrins that are released when antimicrobial agents kill the microorganism.

Although a number of investigators have reported enigmatic porphyria in patients who had no evidence of abnormal enzymes in the heme biosynthesis pathway (Yeung Laiwah et al., Lancet, i:790-792 (1983); Mustajoki, P. and Tenhunen, R., Europ. Journ. of Clin. Invest., 15:281-284 (1985)), the intrinsic secondary, obligatory porphyria caused by chlamydial infection disclosed herein has neither been described nor hypothesized in the medical literature. This obligatory secondary porphyria clearly is of paramount importance in dealing with chronic systemic chlamydial infections as are seen with intravascular infections caused by Chlamydia pneumoniae.

The diagnosis of chlamydial-associated secondary porphyria is important because of the well known neuropsychiatric manifestations of porphyrrias (Gibson et al., Journal of Pathology and Bacteriology, 71:495-509 (1956); Bonkowsky et al., Seminars in Liver Diseases, 2:108-124 (1982); Brennan et al., International Journal of Biochemistry, 833-835 (1980); Burgoyne et al., Psychotherapy and Psychosomatics, 64:121-131 (1995)). Moreover, chronic exposure to excess porphyrins has been associated with cancer (Kordac V., Neoplasma, 19:135-139 (1972); Lithner et al., Acta Medica Scandinavina, 215:271-274 (1984)). Of particular interest is that infection with Chlamydia pneumoniae has been associated with lung cancer (Cerutti P A., Science, 227:375-381 (1985)).

The diagnosis of genetic porphyria in patients with systemic chlamydial infections is important as these patients may precipitate a severe porphyrin attack when they receive antimicrobial agents to treat their infection. Thus, in order to control the severe porphyria, these patients may require intravenous hematin and/or plasmapheresis in addition to the oral anti-porphyrin agents. In contrast, the diagnosis of chlamydial-associated secondary porphyria may be difficult as the porphyria may be minimal and tissue-specific. The measurement of 24 hour urine porphyrins is not sensitive enough in every case of chlamydial infection to detect the secondary porphyria caused by chlamydial infection.

In view of the foregoing discussion of the etiology of porphyria, one aspect of the invention pertains to methods for differentiating porphyria caused by Chlamydia from that caused by a latent genetic disorder in an individual. The method comprises treating infection by Chlamydia at all stages of its life cycle, using the therapies described in detail elsewhere in this disclosure, and then assessing whether symptoms of porphyria have been reduced. A reduction in the symptoms of porphyria (e.g., biochemical, enzymatic or physical manifestation) are indicative that the porphyria is a secondary porphyria caused by Chlamydia.

The diagnosis of genetic porphyria is most easily done during an acute porphyrin attack as there are porphyrinogen precursors and porphyrins in the blood, urine and stool (Kauppinen et al., British Journal of Cancer, 57:117-120(1988)). The diagnosis of secondary porphyria is not as easy to do as there may not be an abnormal amount of porphyrinogen precursors and porphyrins in the blood, urine, or stool. However, several early enzymes in the pathway for heme biosynthesis can be readily measured in peripheral red blood cell (Percy et al., South African Forensic Medicine Journal, 52:219-222 (1977); Welland et al., Metabolism, 13:232-250 (1964); McColl et al., Journal of Medical Genetics, 19:271-276 (1982)). Specific hereditary porphyrias that can be diagnosed with the measurement of low levels of peripheral red blood cell enzymes are acute intermittent porphyria, congenital erythropoietic porphyria, D-aminolevulinic acid dehydratase deficiency porphyria, and porphyria cutanea tarda. Therefore, elevated porphyrin levels in patients who do not have low levels of these enzymes is suggestive of a non-genetic porphyria, such as chlamydially induced secondary porphyria. For example, in one embodiment, porphyria caused by Chlamydia in an
individual having symptoms associated therewith can be diagnosed by determining the presence and/or amount of obligatory enzymes in heme biosynthesis in red blood cells of the individual. The presence or amount of the obligatory enzyme is compared to a normal patient who does not have porphyria or to an earlier test result in the patient to determine the patient’s porphyria symptoms and/or whether therapy is effective. For example, the presence of ALA synthase and/or PBG deaminase or any of the other known enzymes involved in heme biosynthesis (see Table 7), in abnormal levels (i.e., significant deviation from normal levels in healthy patients who do not have genetic porphyria) is indicative of secondary porphyria.

The diagnosis of chlamydial-associated secondary porphyria may be difficult as the porphyria may be minimal and tissue-specific. The measurement of 24 hour urine or stool porphyrins may not be sensitive enough in many cases of chlamydial infection to detect the secondary porphyria. Here, the diagnosis depends on the fact that if excess porphyrins are reaching the circulation, the precursor red blood cells will absorb these and make heme. Thus, the enzymes for heme biosynthesis in the differentiated red blood cell become elevated and remain elevated for the life of the red cell. This allows the diagnosis of episodic low-level secondary porphyria as is seen with chlamydial infections. Thus, elevated beme synthesis levels can be used to diagnose intracellular porphyria. See Example 7.

As discussed above, some patients having a Chlamydia-induced porphyria do not have abnormal levels of heme precursors. For those patients it may be appropriate to determine the presence of Chlamydia as well as porphyrins in the individual. The presence of both the pathogen and porphyrins (e.g., determined by ELISA assay described below) is indicative of secondary chlamydial porphyrin, rather than a genetic based porphyrin. A proper diagnosis can thus determine the therapeutic regimen needed to treat infection and symptoms of secondary porphyria.

The inventors have discovered the existence of antibodies to the various metabolites of heme biosynthesis, as well as Vitamin B12 (cobalamin), which is molecularly similar to these metabolites, in patients with active systemic infection with C. pneumoniae. The antibodies are primarily IgM; this is similar to the antibody responses to the MOMP of C. pneumoniae in severely symptomatic patients. Example 8 illustrates titers in symptomatic patients with systemic C. pneumoniae infections. The presence of antibodies to Vitamin B12 may have functional significance by decreasing the amount of bioavailable Vitamin B12. Thus, a Chlamydia infection may cause a previously unrecognized secondary Vitamin B12 deficiency. Administration (e.g., intramuscular) of large quantities of Vitamin B12 (1000 to 5000 μg) (e.g., parenteral cobalamin therapy) creates large amounts of Vitamin B12 available for binding to the native receptors of antibodies with an affinity for Vitamin B12, thereby saturating these anti-Vitamin B12 antibodies and increasing the amount of bioavailable circulating Vitamin B12.

The previously unknown fact that the body produces antibodies to porphyrins makes it possible to diagnose the presence of porphyrins in a patient or animal by determining the presence of anti-porphyrin antibodies. The inventors have developed a method in which IgM and IgG antibodies to porphyrins can be measured with an ELISA method. This has been shown to be a much more accurate method to determine the chronic presence of porphyrins.

Porphyrins can also be used to create monoclonal and polyclonal antibodies using standard methods known to any one skilled in the field. These antibodies can be used in a variety of diagnostic assays and anti-porphyrin therapeutic strategies.

Treatment of Chlamydia infection may exacerbate secondary porphyria by increasing the metabolism of cryptic Chlamydia or by accelerating the death of infected cells with elevated intracellular porphyrin levels.

Once secondary porphyria is diagnosed, chlamydial infection and symptoms associated with porphyria can be treated. The following therapeutic regimen is aimed at controlling the chlamydial-associated secondary/obligatory porphyria, symptoms of which can actually increase during antimicrobial therapy of the chlamydial infection. This porphycric reaction to antimicrobial therapy should be recognized as such and differentiated from the expected cytokine-mediated immune response precipitated by antigen dump during anti-chlamydial therapy. These obligatory and secondary chlamydial metabolic disorders are treated by specific diets and a combination of pharmacological agents, each directed at different aspects of the metabolic disorders. For example, chlamydial-induced porphyria can be treated with a specific
antiporphyric diet and a combination of antiporphyric agents, each directed at different aspects of porphyrins/porphyria. For purposes of this invention, the term "antiporphyric agent(s)" is intended to embrace any of the therapies described herein for management of porphyria. In addition to the antiporphyric diet and antiporphyric agents, the patient may require intravenous glucose and hematin, renal dialysis, and/or plasmaphoresis, particularly for those patients having both genetic porphyria and secondary porphyria induced by a chlamydial infection. Suitable diets and antiporphyric agents are described in detail below.

C. Therapies to Enhance Cellular Function

Glucose is an important source of cellular energy. Glucose levels can be enhanced by diet and through vitamin supplements as described below.

A high carbohydrate diet should be maintained to promote production of glucose (Pierach et al., Journal of the American Medical Association, 257:60-61 (1987)). Approximately 70% of the caloric intake should be in the form of complex carbohydrates such as bread, potato, rice and pasta. The remaining 30% of the daily diet should comprise protein and fat, which should ideally be in the form of fish or chicken. Red meats, including beef, dark turkey, tuna and salmon, contain tryptophan. Increased levels of tryptophan in the liver inhibit the activity of phosphoenol pyruvate carboxykinase with consequent disruption of gluconeogenesis. This accounts for the abnormal glucose tolerance seen in porphyria. Increased plasmic concentrations of tryptophan also enhances tryptophan transport into the brain. The concentration of tryptophan in the brain is the rate-limiting factor for the synthesis of the neurotransmitter 5-hydroxytryptamine (5-HT, serotonin). Serotonin is synthesized by the endothelium of brain capillaries for circulating tryptophan. Thus, increased concentrations of tryptophan in the brain would be expected to enhance production of serotonin and its metabolic, 5-hydroxyindole-acetic acid (5HIAA). Acute increases in serotonin turnover in the brain are followed by vascular and metabolic changes which include decreases in glucose consumption, disturbances in EEG tracings, and decreases in the postischemic neurological score. In addition, while serotonin increases brain perfusion on a single injection, repetitive administration initially opens the blood-brain barrier and subsequently induces vasoconstriction. It is likely that any transient opening of the blood-brain barrier by serotonin could allow circulating substrates such as ALA and PBG, if present, to enter the central nervous system. As would be expected from the location of serotonin receptors and from the barrier function of the endothelium of cerebral arteries, the constricting effect of serotonin is amplified in cerebral arteries where endothelium is damage or removed. Damaged endothelial cells, as would be expected with chlamydial infection, would no longer have operational catabolic processes for serotonin. This would be particularly true in the event of depleted ATP as caused by chlamydial infection. This means that increased concentrations of serotonin will reach the smooth muscle layer of the cerebral vessels and cause more constriction. Finally, serotonin is also stored in blood platelets. Because blood platelets do not adhere and aggregate under normal conditions, they do not release serotonin when the vessel lumen is intact. However, if the vessel lumen is altered by chlamydial infection, platelet deposition and release of serotonin can occur.

Another adverse effect of increased serotonin levels due to porphyria is seen with nervous tissues. Sympathetic nerve endings store serotonin taken up from the circulation. These serotoninergic neurons form plexuses around brain vessels where they are likely to liberate their serotonin contents when subjected to cellular lysis from any cause including ischemia, free-radical ionizing damage to cell membranes, and/or chlamydial infection.

In rats, elevated circulating tryptophan has been shown to produce structural alteration of brain astrocytes, oligodendroglia, and neurons, as well as degeneration of Purkinje cells and wasting of axons. Similar neuropathological alterations have been reported in patients with acute porphyria. Elevated tryptophan levels in plasma and brain have been associated with human encephalopathy. Finally, serotonin is also recognized as an active neurotransmitter in the gastrointestinal tract. The pharmacologic effects of serotonin in the central nervous system and gastrointestinal tract resemble the neurological manifestations of acute porphyrin attacks. In fact, administration of either tryptophan or serotonin to humans have been reported to cause severe abdominal pain, psychomotor disturbances, nausea, and dysuria; all of which are symptoms of acute porphyria.

Sucrose and fructose should be avoided (Bottomly et al., American Journal of Clinical Pathology, 76:133-139 (1981))