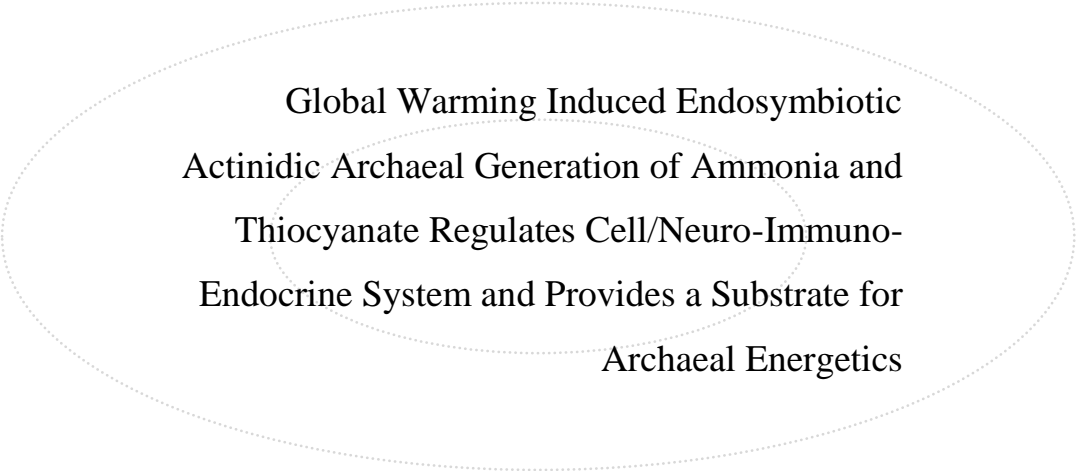


Chapter 8



Global Warming Induced Endosymbiotic
Actinidic Archaeal Generation of Ammonia and
Thiocyanate Regulates Cell/Neuro-Immuno-
Endocrine System and Provides a Substrate for
Archaeal Energetics

Introduction

Climate change and related stress leads to increased porphyrin synthesis leading onto porphyrinuria and porphyria. The stimulus for porphyrin synthesis comes from heme deficiency. Heme suppresses ALA synthase. Stress induces heme oxygenase which converts heme to carbon monoxide and bilirubin. Thus heme is depleted from the system. Heme oxygenase is induced by environmental stress. Climatic changes of global warming and ice age can induce heme oxygenase. Heme oxygenase is also induced by EMF pollution of the environment. Thus there is increased porphyrin synthesis from succinyl CoA and glycine. The porphyrins can self organize to form macromolecular structures which can self replicate to form a porphyrin organism.¹ The photon induced transfer of electrons along the macromolecule can lead to light induced ATP synthesis. The porphyrins can form a template on which RNA and DNA can form generating viroids. The porphyrins can also form a template on which prions can form. They all can join together - RNA viroids, DNA viroids, prions - to form primitive archaea. Thus the archaea are capable of self replication on porphyrin templates. The self replicating archaea can sense gravity which gives rise to consciousness. They can also sense the anti-gravity fields which gives rise to the unconscious brain. Thus there can be both self replicating archaea and anti-archaea regulating the conscious and unconscious brain. Thus the climate change stress mediated increased porphyrin synthesis leads to prefrontal cortex atrophy, cerebellar dominance, cerebellar cognitive affective disorder, quantal perception and Neanderthalisation of the population. The porphyrions are self replicating supramolecular organisms which forms the precursor template on which the viroids, prions and nanoarchaea originate. Stress induced template directed abiogenesis of porphyrions, prions, viroids and archaea is a

continuous process and can contribute to changes in brain structure and behavior as well as disease process.

Actinidic archaea has also been related to the pathogenesis of schizophrenia, malignancy, metabolic syndrome x, autoimmune disease and neuronal degeneration. Actinidic archaea use cholesterol as a carbon and energy source.¹⁻⁹ Archaeal cholesterol catabolism mediated by archaeal cholesterol oxidase can produce cholesterol ring oxidation to generate pyruvate. Pyruvate is converted to glutamate by the enzyme serum glutamate pyruvate transaminase. The glutamate gets acted upon by glutamate dehydrogenase to generate ammonia. Archaeal urease can act upon urea generating ammonia and thiocyanate. Ammonia and thiocyanate serves the purpose of cellular and neuroimmune endocrine regulation. The archaea are ammonia oxidizing and can use ammonia for their energetics. The archaeal urease activity related ammonia and thiocyanate synthesis as well as cholesterol oxidase activity generating pyruvate and ammonia was studied in schizophrenia, malignancy, metabolic syndrome x, autoimmune disease and neuronal degeneration.

Materials and Methods

The following groups were included in the study: - endomyocardial fibrosis, Alzheimer's disease, multiple sclerosis, non-Hodgkin's lymphoma, metabolic syndrome x with cerebrovascular thrombosis and coronary artery disease, schizophrenia, autism, seizure disorder, Creutzfeldt Jakob's disease and acquired immunodeficiency syndrome. There were 10 patients in each group and each patient had an age and sex matched healthy control selected randomly from the general population. The blood samples were drawn in the fasting state before treatment was initiated. Plasma from fasting heparinised blood was used and the experimental protocol was as follows (I) Plasma+phosphate buffered

saline, (II) same as I+cholesterol substrate, (III) same as II+rutile 0.1 mg/ml, (IV) same as II+ciprofloxacin and doxycycline each in a concentration of 1 mg/ml. Cholesterol substrate was prepared as described by Richmond.¹⁰ Aliquots were withdrawn at zero time immediately after mixing and after incubation at 37 °C for 1 hour. The following estimations were carried out: - Cytochrome F420, hydrogen peroxide, pyruvate, ammonia, glutamate, thiocyanate and urease activity.¹¹⁻¹³ Cytochrome F420 was estimated fluorimetrically (excitation wavelength 420 nm and emission wavelength 520 nm). Informed consent of the subjects and the approval of the ethics committee were obtained for the study. The statistical analysis was done by ANOVA.

Results

Plasma of control subjects showed increased levels of the above mentioned parameters with after incubation for 1 hour and addition of cholesterol substrate resulted in still further significant increase in these parameters. The plasma of patients showed similar results but the extent of increase was more. The addition of antibiotics to the control plasma caused a decrease in all the parameters while addition of rutile increased their levels. The addition of antibiotics to the patient's plasma caused a decrease in all the parameters while addition of rutile increased their levels but the extent of change was more in patient's sera as compared to controls. The results are expressed in tables 1-4 as percentage change in the parameters after 1 hour incubation as compared to the values at zero time. The results show increased archaeal urease activity generating ammonia and thiocyanate in the disease states. It also shows increased cholesterol ring oxidase activity generating pyruvate. The pyruvate is converted by SGPT to glutamate. Glutamate dehydrogenase converts glutamate to ammonia. There is generation of ammonia by archaeal urease and cholesterol oxidase activity.

Table 1 *Effect of rutile and antibiotics on cytochrome F420.*

Group	CYT F420 % (Increase with Rutile)		CYT F420 % (Decrease with Doxy+Cipro)	
	Mean	±SD	Mean	±SD
Normal	4.48	0.15	18.24	0.66
Schizo	23.24	2.01	58.72	7.08
Seizure	23.46	1.87	59.27	8.86
AD	23.12	2.00	56.90	6.94
MS	22.12	1.81	61.33	9.82
NHL	22.79	2.13	55.90	7.29
DM	22.59	1.86	57.05	8.45
AIDS	22.29	1.66	59.02	7.50
CJD	22.06	1.61	57.81	6.04
Autism	21.68	1.90	57.93	9.64
EMF	22.70	1.87	60.46	8.06
F value	306.749		130.054	
P value	< 0.001		< 0.001	

Table 2 *Effect of rutile and antibiotics on pyruvate and glutamate.*

Group	Pyruvate % change (Increase with Rutile)		Pyruvate % change (Decrease with Doxy+Cipro)		Glutamate (Increase with Rutile)		Glutamate (Decrease with Doxy+Cipro)	
	Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD
Normal	4.34	0.21	18.43	0.82	4.21	0.16	18.56	0.76
Schizo	20.99	1.46	61.23	9.73	23.01	2.61	65.87	5.27
Seizure	20.94	1.54	62.76	8.52	23.33	1.79	62.50	5.56
AD	22.63	0.88	56.40	8.59	22.96	2.12	65.11	5.91
MS	21.59	1.23	60.28	9.22	22.81	1.91	63.47	5.81
NHL	21.19	1.61	58.57	7.47	22.53	2.41	64.29	5.44
DM	20.67	1.38	58.75	8.12	23.23	1.88	65.11	5.14
AIDS	21.21	2.36	58.73	8.10	21.11	2.25	64.20	5.38
CJD	21.07	1.79	63.90	7.13	22.47	2.17	65.97	4.62
Autism	21.91	1.71	58.45	6.66	22.88	1.87	65.45	5.08
EMF	22.29	2.05	62.37	5.05	21.66	1.94	67.03	5.97
F value	321.255		115.242		292.065		317.966	
P value	< 0.001		< 0.001		< 0.001		< 0.001	

Table 3 *Effect of rutile and antibiotics on hydrogen peroxide and ammonia.*

Group	H ₂ O ₂ % (Increase with Rutile)		H ₂ O ₂ % (Decrease with Doxy+Cipro)		Ammonia % (Increase with Rutile)		Ammonia % (Decrease with Doxy+Cipro)	
	Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD
Normal	4.43	0.19	18.13	0.63	4.40	0.10	18.48	0.39
Schizo	22.50	1.66	60.21	7.42	22.52	1.90	66.39	4.20
Seizure	23.81	1.19	61.08	7.38	22.83	1.90	67.23	3.45
AD	22.65	2.48	60.19	6.98	23.67	1.68	66.50	3.58
MS	21.14	1.20	60.53	4.70	22.38	1.79	67.10	3.82
NHL	23.35	1.76	59.17	3.33	23.34	1.75	66.80	3.43
DM	23.27	1.53	58.91	6.09	22.87	1.84	66.31	3.68
AIDS	23.32	1.71	63.15	7.62	23.45	1.79	66.32	3.63
CJD	22.86	1.91	63.66	6.88	23.17	1.88	68.53	2.65
Autism	23.52	1.49	63.24	7.36	23.20	1.57	66.65	4.26
EMF	23.29	1.67	60.52	5.38	22.29	2.05	61.91	7.56
F value	380.721		171.228		372.716		556.411	
P value	< 0.001		< 0.001		< 0.001		< 0.001	

Table 4 *Effect of rutile and antibiotics on archaeal urease and thiocyanate.*

Group	Thiocyanate % (Increase with Rutile)		Thiocyanate % (Decrease with Doxy+Cipro)		Archaeal urease (Increase with Rutile)		Archaeal urease (Decrease with Doxy+Cipro)	
	Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD
Normal	4.40	0.10	18.48	0.39	4.45	0.14	18.25	0.72
Schizo	22.52	1.90	66.39	4.20	23.01	1.69	59.49	4.30
Seizure	22.83	1.90	67.23	3.45	22.67	2.29	57.69	5.29
AD	23.67	1.68	66.50	3.58	23.26	1.53	60.91	7.59
MS	22.38	1.79	67.10	3.82	22.83	1.78	59.84	7.62
NHL	23.34	1.75	66.80	3.43	22.84	1.42	66.07	3.78
DM	22.87	1.84	66.31	3.68	23.40	1.55	65.77	5.27
AIDS	23.45	1.79	66.32	3.63	23.23	1.97	65.89	5.05
CJD	23.17	1.88	68.53	2.65	23.46	1.91	61.56	4.61
Autism	23.20	1.57	66.65	4.26	22.61	1.42	64.48	6.90
EMF	22.29	2.05	61.91	7.56	23.73	1.38	65.20	6.20
F value	372.716		556.411		391.318		257.996	
P value	< 0.001		< 0.001		< 0.001		< 0.001	

Discussion

There was increase in cytochrome F420 indicating archaeal growth. The archaea can synthesize and use cholesterol as a carbon and energy source.¹⁴⁻¹⁶ The archaeal origin of the enzyme activities was indicated by antibiotic induced suppression. The study indicates the presence of actinide based archaea with an alternate actinide based enzymes or metalloenzymes in the system as indicated by rutile induced increase in enzyme activities.¹⁴⁻¹⁶ The archaeal cholesterol oxidase activity was increased resulting in generation of pyruvate and hydrogen peroxide.¹⁴⁻¹⁶ The pyruvate gets converted to glutamate by serum glutamate pyruvate transaminase. Glutamate is acted upon by glutamate dehydrogenase generating alpha ketoglutarate and ammonia. The archaeal urease acts upon urea as the substrate and generates thiocyanate and ammonia. The archaeal urease and cholesterol oxidase are actinide dependent and activated by rutile. They are suppressed by antibiotics. Ammonia and thiocyanate serves the purpose of cellular and neuro-immuno-endocrine regulation. The archaea are ammonia oxidizing and can use ammonia for their energetics.

The archaeal ammonia can regulate brain function. The ammonia can function as a synaptic gasotransmitter. Ammonia can stimulate GABA receptors at high levels and NMDA receptors at low levels. Thus ammonia has a biphasic action in that it can modulate both GABA and NMDA receptors. Thus ammonia can regulate the NMDA/GABA thalamo-cortico-thalamic pathway mediating conscious perception. Ammonia is involved in the pathogenesis of schizophrenia and mood disorders. Ammonia can stimulate membrane sodium potassium ATPase activity. Membrane sodium potassium ATPase when stimulated leads to decrease in intracellular calcium and increase in intracellular magnesium resulting in modulation of multiple neurotransmitter systems. The neurotransmitter release from the presynaptic vesicles is calcium dependent.¹⁷⁻²⁰

Membrane sodium potassium ATPase uses 80 percent of the mitochondrial synthesized ATP. Elevated ammonia levels results in a hyperactive membrane sodium potassium ATPase and exhausts all ATP reserves. The mitochondria get fatigued leading on to mitochondrial dysfunction. Ammonia can open the mitochondrial permeability transient, produce cytochrome C release and activate the caspase cascade. Cyanide ion released by urease action can produce cytochrome C oxidase inhibition and mitochondrial dysfunction. Mitochondrial dysfunction can lead onto neuronal degeneration.¹⁷⁻²⁰

Ammonia inhibits insulin release from beta cells contributing to the diabetic state. The hyperactive membrane sodium potassium ATPase due to increased ammonia levels produces increased ATP usage and mitochondrial fatigue. The mitochondrial PT pore opening produce by ammonia and related mitochondrial dysfunction leads onto inefficient energetics and metabolic syndrome x. The archaeal urease activity generates the thiocyanate ion from urea. Cyanide inhibits mitochondrial cytochrome C oxidase and produces mitochondrial dysfunction. Cyanide toxicity has been related to mucoid angiopathy implicated in coronary artery disease and strokes. Cyanide toxicity leads to pancreatic dysfunction and diabetes mellitus manifesting as chronic calcific pancreatitis. Cyanide toxicity can also lead to multinodular goiter and endomyocardial fibrosis. Thus the generation of thiocyanate from urea by the activity of archaeal urease can contribute to a cardiac endocrine syndrome. Thiocyanate can also modulate protein function and structure by binding to proteins. This produces thiocynation of proteins. Thus thiocyanate can modulate cell function.¹⁸⁻²³ Ammonia can function as a mutagen and contribute to alteration in DNA function.

Ammonia can also modulate immune function. It can alter T cell and B cell function. Ammonia can be immunostimulatory or immunosuppressive

depending on its levels. Ammonia can contribute to the genesis of autoimmune disease.²⁴⁻²⁶ Ammonia can also produce cell proliferation and oncogenic transformation as exemplified in gastric carcinoma and hepatomas. During autophagy, portions of the cytoplasm are sequestered into autophagosomes and digested by lysosomal hydrolases. Massive autophagy can be induced in mammalian tissues in a coordinated fashion through nutrient deprivation, which has prompted the search of soluble metabolites that can stimulate autophagy. Ammonia, which is generated as a by-product of glutaminolysis, has been identified as a diffusible factor that stimulates autophagy. Intriguingly, cancer cells increase the rate glutaminolysis and the interstitial fluid of cancers contains higher-than-normal physiological concentrations of ammonia, suggesting a previously unknown pathway through which tumor cells can condition their microenvironment.^{27, 28}

Thus ammonia and thiocyanate produced by endosymbiotic archaeal metabolism modulate neural transmission, metabolic/mitochondrial function, immunity, cell death, cell proliferation and endocrine/pancreatic function. Thus the archaea can use ammonia as a signaling molecule to produce neuro-immune-metabolic-endocrine integration. The archaea are ammonia oxidizing and can use ammonia for their energetics.²⁹ The archaeal utilization of ammonia as a signalling molecule and for energetics may be a remnant of ammonia based primitive archaeal life forms and metabolism. Liquid ammonia can replace water as a solvent for life to originate.

References

- [1] Hanold D., Randies, J. W. (1991). Coconut cadang-cadang disease and its viroid agent, *Plant Disease*, 75, 330-335.
- [2] Valiathan M. S., Somers, K., Kartha, C. C. (1993). *Endomyocardial Fibrosis*. Delhi: Oxford University Press.

- [3] Edwin B. T., Mohankumaran, C. (2007). Kerala wilt disease phytoplasma: Phylogenetic analysis and identification of a vector, *Proutista moesta*, *Physiological and Molecular Plant Pathology*, 71(1-3), 41-47.
- [4] Kurup R., Kurup, P. A. (2009). *Hypothalamic digoxin, cerebral dominance and brain function in health and diseases*. New York: Nova Science Publishers.
- [5] Eckburg P. B., Lepp, P. W., Relman, D. A. (2003). Archaea and their potential role in human disease, *Infect Immun*, 71, 591-596.
- [6] Smit A., Mushegian, A. (2000). Biosynthesis of isoprenoids via mevalonate in Archaea: the lost pathway, *Genome Res*, 10(10), 1468-84.
- [7] Adam Z. (2007). Actinides and Life's Origins, *Astrobiology*, 7, 6-10.
- [8] Schoner W. (2002). Endogenous cardiac glycosides, a new class of steroid hormones, *Eur J Biochem*, 269, 2440-2448.
- [9] Davies P. C. W., Benner, S. A., Cleland, C. E., Lineweaver, C. H., McKay, C. P., Wolfe-Simon, F. (2009). Signatures of a Shadow Biosphere, *Astrobiology*, 10, 241-249.
- [10] Richmond W. (1973). Preparation and properties of a cholesterol oxidase from nocardia species and its application to the enzymatic assay of total cholesterol in serum, *Clin Chem*, 19, 1350-1356.
- [11] Snell E. D., Snell, C. T. (1961). *Colorimetric Methods of Analysis*. Vol 3A. New York: Van Nostrand.
- [12] Glick D. (1971). *Methods of Biochemical Analysis*. Vol 5. New York: Interscience Publishers.
- [13] Colowick, Kaplan, N. O. (1955). *Methods in Enzymology*. Vol 2. New York: Academic Press.
- [14] Van der Geize R., Yam, K., Heuser, T., Wilbrink, M. H., Hara, H., Anderton, M. C. (2007). A gene cluster encoding cholesterol catabolism in a soil actinomycete provides insight into Mycobacterium tuberculosis survival in macrophages, *Proc Natl Acad Sci USA*, 104(6), 1947-52.
- [15] Francis A. J. (1998). Biotransformation of uranium and other actinides in radioactive wastes, *Journal of Alloys and Compounds*, 271(273), 78-84.

- [16] Probian C., Wülfing, A., Harder, J. (2003). Anaerobic mineralization of quaternary carbon atoms: Isolation of denitrifying bacteria on pivalic acid (2,2-Dimethylpropionic acid), *Applied and Environmental Microbiology*, 69(3), 1866-1870.
- [17] Jones EA. (2002). Ammonia, the GABA neurotransmitter system, and hepatic encephalopathy. *Metab Brain Dis.* 17(4), 275-81.
- [18] Veauvy CM, Wang Y, Walsh PJ, Pérez-Pinzón MA. (2002). Comparison of the effects of ammonia on brain mitochondrial function in rats and gulf toadfish. *Am J Physiol Regul Integr Comp Physiol.* 283(3), R598-603.
- [19] Norenberg MD, Rama Rao KV, Jayakumar AR. (2004) Ammonia neurotoxicity and the mitochondrial permeability transition. *J Bioenerg Biomembr*, 36(4): 303-7.
- [20] Xue Z, Li B, Gu L, Hu X, Li M, Butterworth RF, Peng L. (2010). Increased Na, K-ATPase alpha2 isoform gene expression by ammonia in astrocytes and in brain in vivo. *Neurochem Int.*, 57(4): 395-403.
- [21] Wu J., Wu G., Wu L. (2008). Thiocyanation of Aromatic and Heteroaromatic Compounds Using Ammonium Thiocyanate and I₂O₅. *ChemInform*, 39 (51).
- [22] Jensen P., Wilson M. T., Aasa R., Malmström B. G. (1984). Cyanide inhibition of cytochrome c oxidase. A rapid-freeze e.p.r. investigation. *Biochem J*, 224(3), 829-837.
- [23] Emmanuel B., Thompson J. R., Christopherson R. J., Milligan L. P., Berzins, R. (1982). Interrelationships between urea, ammonia, glucose, insulin and adrenaline during ammonia-urea toxicosis in sheep (*Ovis aries*). *Comparative Biochemistry and Physiology Part A: Physiology*, 72(4), 697-702.
- [24] Liu, C. H., Chen, J. C. (2004). Effect of ammonia on the immune response of white shrimp *Litopenaeus vannamei* and its susceptibility to *Vibrio alginolyticus*. *Fish & Shellfish Immunology*, 16(3), 321-334.
- [25] Cheng W., Hsiao I. S., Chen J. C. (2004). Effect of ammonia on the immune response of Taiwan abalone *Haliotis diversicolor supertexta* and its susceptibility to *Vibrio parahaemolyticus*. *Fish & Shellfish Immunology*, 17, 193e20.

- [26] von Borell E, Özpınar A, Eslinger KM, et al. (2007). Acute and prolonged effects of ammonia on hematological variables, stress responses, performance, and behavior of nursery pigs. *J Swine Health Prod*, 15(3), 137-145.
- [27] Mariño G., Kroemer G. (2010). Ammonia: A Diffusible Factor Released by Proliferating Cells That Induces Autophagy. *Sci. Signal.* 3, pe19.
- [28] Tsujii M, Kawano S, Tsuji S, Takei Y, Tamura K, Fusamoto H, Kamada T. (1995). Mechanism for ammonia-induced promotion of gastric carcinogenesis in rats. *Carcinogenesis*, 16(3), 563-6.
- [29] You J., Das A., Dolan E. M., Hu, Z. (2009). Ammonia-oxidizing archaea involved in nitrogen removal. *Water Research*, 43(7), 1801-1809.