

# **Chapter 21**

**Endosymbiotic Actinidic Archaeal Synthesis of  
Short Chain Fatty Acid Butyrate and Propionate  
from Cholesterol Regulates Cellular Function**

## 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.<sup>1</sup> 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.

Endomyocardial fibrosis along with the root wilt disease of coconut is also endemic to Kerala with its radioactive actinide beach sands. Actinides like rutile as well as organisms like phytoplasmas and viroids have been implicated in the etiology of these diseases.<sup>1-4</sup> Short chain fatty acids butyrate and propionate has been related to the pathogenesis of schizophrenia, malignancy, metabolic syndrome X, autoimmune disease and neuronal degeneration.<sup>4</sup> The actinidic archaea by cholesterol side chain oxidation generates butyrate and propionate which can regulate immune, metabolic, neural and genomic function. The possibility of short chain fatty acids butyrate and propionate synthesis by actinide based primitive organism like archaea with a mevalonate pathway and cholesterol catabolism was considered in this study.<sup>5-8</sup>

## 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, and (IV) same as II+ciprofloxacin and doxycycline each in a concentration of 1 mg/ml. Cholesterol substrate was prepared as described by Richmond.<sup>10</sup> 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, butyrate and propionate.<sup>11-13</sup> Cytochrome F420 was estimated fluorimetrically (excitation wavelength 420 nm and emission wavelength 520 nm). Butyrate and propionate were estimated by HPLC method. 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 rutil increased their levels. The addition of antibiotics to the patient's plasma caused a decrease in all the parameters while addition of rutil increased their levels but the extent of change was more in patient's sera as compared to controls. The results are expressed in tables 1-2 as percentage change in the parameters after 1 hour incubation as compared to the values at zero time.

**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 butyrate and propionate generation from cholesterol.

Group	Butyrate% change (Increase with Rutile)		Butyrate% change (Decrease with Doxy+Cipro)		Propionate % change (Increase with Rutile)		Propionate % change (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	

## Discussion

There was increase in cytochrome F420 indicating archaeal growth. The archaea can synthesize and use cholesterol as a carbon and energy source.<sup>6, 14</sup> 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.<sup>15, 16</sup> There was an increase in cholesterol side chain oxidation by actinidic archaea. This is indicated by the presence of increasing butyrate and propionate in the system. The cholesterol side chain oxidation generating butyrate and propionate is inhibited by antibiotics and stimulated by rutile. The archaea can undergo magnetite and calcium carbonate mineralization and can exist as calcified nanoforms.<sup>17</sup>

Archaeal butyrate can produce histone deacetylase inhibition resulting in endogenous retroviral (HERV) reverse transcriptase and integrase expression. This can integrate the HERV RNA complementary DNA into the noncoding region of eukaryotic noncoding DNA using HERV integrase as has been described for borna and ebola viruses.<sup>19</sup> The noncoding DNA is lengthened by integrating HERV RNA complementary DNA with the integration going on as a continuing event. The archaea genome can also get integrated into human genome using integrase as has been described for trypanosomes.<sup>20</sup> The integrated archaea can undergo vertical transmission and can exist as genomic parasites.<sup>19, 20</sup> This increases the length and alters the grammar of the noncoding region producing memes or memory of acquired characters as well as eukaryotic speciation and individuality.<sup>21</sup> The HERV RNA complementary DNA can function as jumping genes producing a dynamic genome important in storage of synaptic information, HLA gene expression and developmental gene expression. The HERV RNA can regulate mRNA function by RNA interference.<sup>18</sup> The phenomena of RNA interference can modulate T cell and B

cell function, insulin signaling lipid metabolism, cell growth and differentiation, apoptosis, neuronal transmission and euchromatin / heterochromatin expression.

Butyrate and propionate are histone deacetylase inhibitors. HDAC inhibitors like butyrate can inhibit cell proliferation. Butyrate is used in the treatment of several malignant neoplasms. Butyrate can also modulate protein folding and function. Butyrate can correct the unfolded protein response. Butyrate is thus used in the treatment of disorders with unfolded protein response. This includes disorders like neuronal degeneration and trinucleotide repeat disease.

Butyrate by modulating protein folding is of use in the management of metabolic syndrome X and insulin resistance syndromes. Butyrate and propionate can bind to lymphocyte GPCR receptors. Butyrate and propionate is immunosuppressive and is used in the treatment of autoimmune disease. Archaeal butyrate and propionate deficiency can lead to immune activation and autoimmune disease.

Butyrate can be converted to the inhibitory gamma aminobutyric acid or GABA or the excitatory beta hydroxybutyric acid. Thus butyric acid metabolites can regulate the NMDA/GABA thalamocorticothalamic pathway mediating conscious perception. Butyrate coma has been described. Butyrate can also modulate gene expression related to multiple neurotransmitter systems. Butyrate and propionate are involved in the genesis of neuropsychiatric disorders. Intraventricular propionate has been related to autism and schizophrenia.<sup>22-28</sup>

Thus short chain fatty acids butyrate and propionate can regulate cell death and cell proliferation through modulation of unfolded protein response and HDAC inhibition. It can regulate the insulin receptor and protein function by modulating protein folding. Butyrate and propionate via binding to lymphocyte GPCR receptors can regulate immune function. Butyrate can also modulate several neurotransmitter systems including NMDA/GABA. Butyrate by

producing HDAC inhibition can regulate gene expression. Thus the archaeal short chain fatty acids butyrate and propionate generated by cholesterol side chain oxidation can regulate the neuro-immune-genetic-endocrine-metabolic system as well as the cell cycle. The archaeal short chain fatty acids may play a role in the pathogenesis of malignancy, degenerations, metabolic syndrome X, autoimmune disease and neuropsychiatric disorders.<sup>22-28</sup>

## 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] Vainshtein M., Suzina, N., Kudryashova, E., Ariskina, E. (2002). New Magnet-Sensitive Structures in Bacterial and Archaeal Cells, *Biol Cell*, 94 (1), 29-35.
- [18] Tsagris E. M., de Alba, A. E., Gozmanova, M., Kalantidis, K. (2008). Viroids, *Cell Microbiol*, 10, 2168.
- [19] Horie M., Honda, T., Suzuki, Y., Kobayashi, Y., Daito, T., Oshida, T. (2010). Endogenous non-retroviral RNA virus elements in mammalian genomes, *Nature*, 463, 84-87.
- [20] Hecht M., Nitz, N., Araujo, P., Sousa, A., Rosa, A., Gomes, D. (2010). Genes from Chagas parasite can transfer to humans and be passed on to children. Inheritance of DNA Transferred from American Trypanosomes to Human Hosts, *PLoS ONE*, 5, 2-10.

- [21] Flam F. (1994). Hints of a language in junk DNA, *Science*, 266, 1320.
- [22] Meijer, K., de Vos P., Priebe M. G. (2010) Butyrate and other short-chain fatty acids as modulators of immunity: what relevance for health? *Curr Opin Clin Nutr Metab Care*, 13 (6), 715-21.
- [23] Chakravorty, D., Koide, N., Kato, Y., Sugiyama, T., Mu, M. M., Yoshida, T., Yokochi T. (2000). The inhibitory action of butyrate on lipopolysaccharide-induced nitric oxide production in RAW 264.7 murine macrophage cells. *J Endotoxin Res*, 6 (3), 243-7.
- [24] Marks, P. A., Jiang, X. (2005). Histone deacetylase inhibitors in programmed cell death and cancer therapy. *Cell Cycle*, 4, 549-551.
- [25] Insinga, A., Monestiroli, S., Ronzoni, S., Gelmetti, V., Marchesi, F., Viale, A., Altucci, L., Nervi, C., Minucci, S., Pelicci, P. G. (2005). Inhibitors of histone deacetylases induce tumor-selective apoptosis through activation of the death receptor pathway. *Nat Med*, 11, 71-76.
- [26] Yam, G. H., Gaplovska-Kysela, K., Zuber, C., Roth, J. (2007). Sodium 4-phenylbutyrate acts as a chemical chaperone on misfolded myocilin to rescue cells from endoplasmic reticulum stress and apoptosis. *Invest Ophthalmol Vis Sci*, 48 (4), 1683-90.
- [27] Kim, P. S., Arvan, P. (1998). Endocrinopathies in the family of endoplasmic reticulum (ER) storage diseases: disorders of protein trafficking and the role of ER molecular chaperones. *Endocr Rev*, 19 (2), 173-202.
- [28] Reger, M. A., Henderson, S. T., Hale, C., Cholerton, B., Baker, L. D. (2004). Effects of beta-hydroxybutyrate on cognition in memory-impaired adults. *Neurobiology of Aging*, 25 (3), 311-314.