Human-specific intractable Immune diseases and mitochondrial deterioration

Katsunari Nishihara[t].*

Nishihara Institute, Hara Bldg 3F, 6-2-5 *Roppongi, Minato-ku, Tokyo. Japan*

This paper introduces the concept of environmental energy as well as mitochondrial energy metabolism into conventional life science and medicine, and shows how morphology, physiology, molecular genetics, biomechanics, bacteriology, virology and clinical therapeutic medicine can be integrated to cure intractable immune maladies. The major causes of immune diseases are disclosed as being brought about by intracellular infection of nonpathogenic common enterom icrobes due to absorbing improper environmental energies. Through controlling environmental energy as well as common enteromicrobes infected intracellularly, it has been shown that these maladies occur by deterioration of mitochondrial function via these microbes. Consequently, the author has established stomata-facial and neuro-cranial medicine and has developed new therapeutic methods for the complete curing of intractable immune diseases via diagnosis *exjuvantihus* through treatment of intracellular infection as well as controlling environmental energy. By means of integrated clinical and basic research. the author has disclosed what human specific intractable immune diseases are, including mental illness and cancer. This allows genuine clinical immunology to be established, by which intractable immune maladies are completely cured, if treated in time. The paper also discusses the essential mitochondrial function of cellular life activity to remodel themselves to overcome aging.

Keywords: energy metabolism, genuine clinical immunology, hypophysis, intracellular infection, intractable immune disease, mitochondrial deterioration

1. INTRODUCTION

There are numerous kinds of creatures having different kinds of life systems, i.e., the prokaryote and the eukaryote, the latter in turn comprising monocellular organisms (protozoa) and multicellular organisms. In the latter category there are vertebrate animals and plants, both of which are derived from the Ascidia (i.e., the Urochordata). The difference between them is the locomotive movement of the body of the former and the permanent association with the ground, fixed by roots, of the latter.

In conventional medicine and life sciences not only environmental energy, i.e., substance without mass. but also the energy metabolism of mitochondria in cells as well as the biomechanical energy of an animal's own movement have been largely overlooked. Moreover, the energy conservation law of Robert Mayer (Caneva, 1993) has been neglected. One striking consequence is that in the tiny world of molecular biology a monocellular prokaryote or eukaryote can live under 10000 *g,* whereas multicellular eukaryote mammals die at less than 7 g. The difference between monocellular and multicellular organisms is a circulating intercellular medium, which is influenced by gravitational energy just like tidal currents. The difference between monocellular and multicellular organisms is only the existence of an intercellular medium via the cardiovascular system of animals or the vascular bundle of plants.

In 1944 Schrodinger published his rather well known book ., *What is Life? The Physical Aspect of the Living Celr'* in which he proposed to establish molecu lar biology by means of the physicist's approach. He considered that the most characteristic phenomenon of life is heredity, but did not actually def! ne "what is life?" and overlooked living energy metabolism in life, which is the most important phenomenon associated with it. He did not notice the fact that the essence of living phenomena is the system of remodel ling, which is closely connected with energy metabolism; with that, the living system can overcome aging. Also in conventional medicine, the essential rôle of mitochondria in human body cells has been essentially completely overlooked. The mitochondria, having their own haploid genes. are presumed to be derived from the archetypical prokaryote, therefore they can be seen as a relic of aerobic archaea. Prokaryotes generate energy for their own life system; however,

t Dr Nishihara is also President of the Japanese Association for Therapeutics of Immune Diseases.
* E-mail: nishihara-ken@a.email.ne.jp

Journal ojBiological Physics and Chemistry 10 (2010) 135-144
Received 19 October 2010; *accepted* 15 December 20 I0

multicellular eukaryote mammals generate 90% of their life energy in mitochondria and only 10% by glycolysis in the cytoplasm (Lehninger, 1964). By glycolysis, glucose is resolved into pyruvates, which are metabolized through the TCA (Krebs) cycle in mitochondria for *de novo* synthesis ofATP.

Without mitochondrial energy metabolism the life system of mammals could never exist. Multicellular mammalian life, which is integrated as a unified system, is completely dependent upon tremendous numbers of mitochondria in the 60 x 10^{12} cells in the body. In order to more deeply understand health, maladies and the life span of mammals, consideration of the mitochondrial condition in all the cells is an ineluctable necessity. Surveying the human history of overcom ing contagious and infectious diseases as well as the history of transition in therapeutic medicine applied to modern maladies, the present author has revealed that intractable immune diseases, including psychiatric disorders and carcinomas, are severe cases of maladies that have been known since 50 years as opportunistic infections or autotoxic diseases, histiocytosis, granulomatosis or sarcoidosis, which have been brought about by nonpathogenic common enteromicrobes. which usually infect intracellularly. Clinical research revealing and curing "intractable" immune diseases has already been reported in several papers by the author (Nishihara. 2008a, 2009a and b). In conventional medicine very few clinicians pay attention to mitochondrial diseases or mitochondrial dysfunction, of which mitochondrial mutation might be the initial observable in diseased cells. The life system of cells in mammals depends completely upon mitochondrial ability, consequently mammalian health and aging as well as lifespan depend completely upon the condition of the mitochondria-whether healthy or deteriorated.

Rickettsia, mycoplasma and chlamydia are contemporary living bacteria that resemble archaea or mitochondria. They live solely as a parasite in the cytoplasma of mammalian cells,just like the mitochondria. Therefore. they can only infect intracellularly via leukocytes (granulocytes). 1ntracellu larly contam inated granulocytes disseminate these bacteria into tissue or organ cells. These bacteria and viruses are so weakly toxic that they can infect intracellularly in apparently. In recent basic medicine, namely bacteriology, virology and pathology. as well as in clinical therapeutic medicine, no one has considered intracellular infection by nonpathogenic common enteromicrobes, although they have been known as opportunistic infections for 50 years. In contrast, infections by pathogenic microbes are so toxic that when they contact an animal medium, maladies ensue immediately. Nevertheless, in intracellular infection by common entero eria mitochondrial deterioration occurs in various tissue r organ cells in patients.

From clinical therape -j studies for intractable immune diseases (including psychiatric disorders and carcinomas), the author onsiders human diseases and healthy life as well as aging from the viewpoints of energy, biomechanics and milochondrial deterioration (Nishihara and Tanaka, 1996: ishihara, 1997. 1999, 2000,200 I, 2003a,b, 2004b. 2005, 2006, 2007a.b: Tache and Morley, 1989).

2. THE LIFE SYSTEM OF MAMMALIA AND THE FUNCTION OF MITOGIONDRIA

In conventional medicine and life science the most important concepts for life, namely not only the energy metabolism of mitochondria. but also the homeostatic environmental energy system and the biomechanical energy system of animal behaviour, have been essentially completely overlooked. By introducing these features into life science and medicine the author has establ ished stomato-facial and neuro-cranial medicine.

Life has not yet been scientifically defined even in the 21 st century. The author defines the essence of eukaryote life as follows: it is based on hydrocolloidal organic substances; i.e., nucleotides, proteins, carbohydrates, lipids etc. with various kinds of minerals encapsulated by phospholipid membranes; a part of or the whole structure remodels itself by means of energy metabolism. This organic system can overcome aging by remodelling; that is, the renewal of tissues or cells. Remodelling concom itant with energy metabolism, i.e., *de novo* synthesis of energy by mitochondria, is essential for life (Nishihara. 2004a). The life of higher animals like vel1ebrates is a circulating system of energy metabolism ultimately driven by solar energy,just like a waterwheel or a windmill as well as terrestrial and lunar gravitational energy fields, which enable energy metabolism via mitochondria coupled with remodelling in the animal body by the energy of metabolized nutrition absorbed from digested food. By this remodelling, animal cells can overcome aging; i.e., decrease entropy (Nishihara, 2006).

Therefore, there is no Iife system for eukaryotes without mitochondria. Th is eircu lating system of energy metabolism necessitates both energy from the cosmos (sunlight) and energy from nutrition (food), the latter being itself ultimately derived from sunlight. The life of higher animals has acceptors of both environmental energy and substance with mass (food). The former are sensory organs and the latter are visceral organs. AJllife phenomena, including morphology, function, remodelling and the genito-urinary system in animals, are completely dependent upon the energy metabolism of their

mitochondria and the genome function of their nucleus. This is the most important point overlooked by Schrodinger in his book "*What is Life?*". Adult Eutheria, multicellular mammalia, have circa 60 x 10^{12} cells, and many specialized organs and structures. They have a complicated, correlated action enabling them to function and move systemically as if constructed from a single cell, like protozoa. The fertilized oosperm cell divides into multiple cells, develops into complicated gastulura, branchilura, neurura, embryos, and foetuses.

In the 21st century, no scientist has apparently seriously considered in medicine or the life sciences how human ontogeny (i.e., development) takes place and how the multicellular human body is constructed from a newborn baby with 3 x 10^{12} cells to an adult with 60 x 10^{12} cells in close correlation, systemically, and a cooperating system among tissue cells and organs, which are themselves constructed from enormous numbers of cells. We have to recognize firstly that the multicellular human body acts as a whole, systemically, as if it were a single-cell organism, a protozoan. Secondly, prokaryotic bacteria cannot form a multicellular organism; only eukaryotes with mitochondria can develop into a multicellular creature. Therefore, mitochondria have the essentially important function to develop a multicellular animal (Nishihara, 2006).

Let us consider the evolution from a protozoan to a multicellular organism. What are the most different structures when one compares them? The most remarkable different parts are: the extracellular cardiovascular circulating systems; the innumerable intracellular organelles, most notably the mitochondria; and the cell membrane, the structure of which is controlled by both nuclear and mitochondrial genes. In the human body, there are various cells at several different evolutionary stages. Leukocytes correspond to the stage of protozoa. Therefore, they can penetrate through small pit holes of blood or lymph vessels with an amoeboid movement. They can invade even the cerebral fluid through weak points of the blood-brain barrier of hypophysis and plexus, and an embryo or foetus through the placenta.

Now, consider the differences between the membrane of leukocytes and that of the cells of other organs. Leukocytes have the MHC (major histocompatibility antigen complex, namely HLA) in its membrane. All cells in the human body, except mature erythrocytes, have MHC genes. However, the genes of the MHC in all cells except leukocytes are dormant (Nishihara, 2004a).

Mitochondria are living organisms in living cells and control all60 x 10^{12} cells as a whole, systemically. in adult mammals, and correlate all the cells in one creature as a united system. Comparing the human body to a nation, the various organs are analogous to states or prefectures, cities or towns, or large communities. Each cell, which has thousands of mitochondria, is like a united community, factory or large, well-run department store. Then what are the numerous mitochondria? Mitochondria are just like the human citizens of a nation-state. Only human citizens can support the nation. Likewise, only vivacious mitochondria can sustain the human body (Nishihara, 2008a). Without healthy mitochondrial function. humans could never maintain their life and health.

The multicellular mammalian body has a unified control system of its multiple organs, in which most of the 60 x 10^{12} cells are organized and which acts as an integrated unit in movement. From the standpoint of reverse calculation in system engineering. in the unified multicellular human body there exist tiny human bodylike creatures just like the citizens of a nation. In the 19th century scholars tried to argue that homunculi live inside human body cells and determine their behaviour. The present author reveals these homunculi to be simply the mitochondria in mammalian cells (Nishihara, 2008a).

Complexion, looks and expression of the face, vigour and vitality, fatigue, tiredness and exhaustion, all of these body states indicate mitochondrial conditions (Nishihara, 2006). We will know of the deterioration of their mitochondria if some important organs lose their function: for example. a heart attack is a dysfunction of mitochondria in cardiac muscles, dementia is a dysfunction of mitochondria in neurons in the cortex of the cerebrum, diabetes mellitus occurs by mitochondrial deterioration in Langerhans' cells, rheumatic arthritis, collagen diseases, and atopic dermatitis occurs by mitochondrial dysfunction in synovial cartilaginous cells, in fibroblasts as well as in connective tissue and subcutaneous tissue cells, respectively, and nephritis is a bacterial infection resulting in a dysfunction of mitochondria in the glomerulus and mesangium. These dysfunctions of mitochondria occur as a result of improper energy metabolism, chronic fatigue and intracellular infection by enterobacteria or viruses. Various kinds of environmental energies (e.g., heat and cold, light and sound, atmospheric pressure, gravity and moisture, including the bio-energy of emotion, religion, spirituality, fear and loss of intimate life, thought and ideology) influence directly or indirectly all mitochondria in the 60 x 10^{12} cells comprising the human body. In addition, energy-consuming activity of animals, namely movement and behaviour, thought and emotion, are supported and influenced reciprocally by mitochondria. By these activities, mitochondria become lively or exhausted. Not only energy fluxes but also toxic substances, intracellular

parasitic microbes and malnutrition influence mitochondria (Nishihara, 2004a).

3. THE OVERALL SYSTEM IN MAMMALIA TO CONTROL MITOCHONDRIA IN WHOLE CELLS DIRECTLY

There should be special acceptors as well as regulators in multicellular organisms, especially mammals, which control energy metabolism over all cells in the body. The special supervising acceptors for systemic energy metabolism in whole cells ill mammals are the suprarenal glands, which are also supervised by the hypophysis (by means of hormones). The suprarenal glands secrete adrenalin as well as mineral- and glyCO-COliicosteroid honnones, which control mitochondrial energy metabolism in whole cells. The control of mitochondrial metabolism in whole cells is the most important system in mammals. The accepting apparatuses for these hypophysis-suprarenal glands systems are whole sensory organs of the somatic system as well as the whole gut visceral system via the epithelial body, all kinds of hormonal glands. the thymus, Waldeyer's lymph-adenoid rings, gut-associated lymphoid tissue (GALT), and whole cells except erythrocytes in the body. This system was adumbrated as Selye's stress theory (Selye, 1937a, 1937b). He was convinced that the major immune functions of animals are carried out by the hypophysis-suprarenal gland system. However, Selye did not know that the hypophysis and suprarenal glands are organs of interpretation of neurons in the limbic system, which are acceptors of various stimuli, and regulators for the energy metabolism of mitochondria in whole cells responding to stimuli that influence the animal body.

The hypophysis secretes not only an adrenocorti-cotropic honnone but various other kinds of hormone as well as cytokines and growth factors, as a regulator responding to the total stimuli impacting the living energy system of an animal. Selye thought that there must be a close direct correlation between the immune system and the central nervous system because the hypophysis is a part of the limbic system of the cerebrum. However, the immune system incorporates cellular digestion as well as a remodelling system conjugated with the energy metabolism of mitochondria, which overcomes the aging of cells (and which is characteristic of living creatures). The limbic system is a structural anatomical system. All neurons in the central nervous system also have their own cellular remodelling system (i.e., the cellular digestion system, namely, the immune system). Therefore, there is no correlation between them directly, but via the stimuli conversion system of the limbic system by which total information from inside the body or outside is integrated via the central nervous system. Subsequently the neurons of the limbic system secrete neurotransmitters, hormones, cytokines and growth factors and transfuse them to the hypophysis via axons (Nishihara, 2006).

What are these total stimuli impinging on the animal? They are entities without mass (i.e., energy) and substance with mass (including oxygen, nutrition, parasitic microbes and toxins). Energy stimuli are heat and cold, thermal gradients and atmospheric pressure, light and electromagnetic waves, sound, moisture and biological energy (i.e., emotion, religion, spirituality, fear, wickedness. and devilishness). Besides the acceptors and the common energy sensors mentioned above, mammals also have receptors and incorporate organs of the branchial respiratory and digestive gut systems of substance with mass, namely nutrition from foods, toxins, parasitic microbes, namely viruses. mycoplasma, bacteria, parasites, toxic proteins and amines. These receptors are the gut absorbing system and tonsilla or lymph-adenoid tissue and thymus. Peyer's patch, epithelial body, cervical sinus, hypophysis and suprarenal glands, which are almost all derived from branchial organs, and the gut-visceral system. The above-mentioned concept of acceptors and regulators has been overlooked by conventional research. The hypophysis and suprarenal glands are acceptors not only for pressure, thermal stimuli, light and sound, but also for various kinds of substances with mass (e.g., parasitic viruses and bacteria as well as toxins).

Peyer's patch is the acceptor for proteins, amines, toxins, viruses and bacteria. For the gut-visceral system, not only sensors for energy, namely the eye, ear and skin, but also the sensors associated with acceptors for substance with mass, namely the sensor of smell, the tongue, taste buds, hypophysis, thymus, GALT and suprarenal glands, which also constitute the incorporation system of nutrition and chemical substances, as well as viruses, bacteria and all other sensors, are subordinate to the gut-visceral system. The hypophysis and suprarenal glands, as well as the cervical sinus, are also receptors of energy. Conventionally, sensory organs are considered to be limited to capture energy. However, all entities, with or without mass, entering the body from outside are accepted as well as absorbed and acknowledged by the somatic or visceral organs and transmitted through the nervous system as well as the blood stream system over whole body's organs and cells.

4. THE CELL RESPIRATION SYSTEM, THE REMODELLING SYSTEM AND THE STIMULI ACCEPTING SYSTEM

The system directly influencing mitochondria is the hypophysis of the suprarenal glands system, which supervises energy metabolism ofmitochondria by means of the cardiovascular circulation system in the whole cells of animals. What are the substances influencing

mitochondria as well as energy metabolism ill the cytoplasma of whole cells? They are:

(I) environmental energy-namely heat and cold, pressure, moisture, light, sound, superson ic waves and electricity:
(2) nutrition, minerals, vitamins, water, oxygen-namely substance with mass for the visceral gut;
(3) toxic substances;
(4) parasitic 111 icrobes-i .e., bacteria, mycoplasma and viruses;
(5) transplanted organs of animals;
(6) biological energy such as spirituality, mind, belief, religions, consciousness, soul and biological stresses.

Initially in Selye's theory stressors, which he called adverse stimuli, included microbial infection. However, in recent therapeutics for intractable immune diseases, bacterial and viral infections are completely overlooked as stressors. For actual animal life, stressors include not only energy as well as nutrition but also toxins, bacteria and viruses, namely substances with mass. Even regarding energy, Selye's conventional stress theory overlooked dynamic force, gravitational energy, muscle movement and thermodynamic energy. Therefore, the hypophysis-suprarenal glands system has to include the all-integrated absorbing and acceptor system of not only energy but also of nutrition as well as of vi ruses and bacteria.

The gut system is the absorbing acceptor system of both energy and substance with mass, the absorption of which is carried out by the blood circulation of the cardiovascu jar system. There are three parts: (I) the branchial respiratory gut; (2) the digestive tract of the gut absorbing system; and (3) the excretory tract, namely the genito-urinary system. This gut absorbing system functions for the cellular remodell ing system in the whole animal body.

There are two remodelling systems, the growth and renewal systems, via the cellular genetic function of nuclei and leukocytes, which are rendered functional by the MHC. The former is cellular metabolism and remodelling in aged cells and the latter is destructive remodelling of tumour cells, intracellularly-infected cells and transplanted nonself organ cells by means of the membrane detector of the MHC (Nishihara, 2004a). All nutrition, minerals, vitamins, oxygen as well as common enteroviruses and bacteria are absorbed from the gut into the blood or leukocytes and delivered to almost all cells via the blood and lymph streams. Concomitantly, microbes absorbed in leukocytes from the M cells of Peyer's patch are disseminated into cells by contaminated leukocytes if the body temperature is lower than 36.5 0C. This gives rise to intracellular infection of organ tissue cells by nonpathogenic enterovimses or bacteria and mycoplasma.

The conventional interpretation of Selye's stress theory has been just half the story because it overlooked the cellular remodelling system as well as intracellular infection of cells in the organism-implying that bacterial or viral contamination in various organ or tissue cells in the entire body occurs. The initial stage of intractable immune diseases starts from intracellular contamination of the hypophysis-suprarenal glands through Waldeyer lymph- adenoid tissue or gut-associated lympho id tissue absorbing enterobacteria or viruses into leukocytes automatically by cooling the gut. After that, dysfunction in secreting adrenocorticotropic hormone takes place, and intracellular infection in various organ cells occurs. This is the cause of intractable immune diseases. Thus, Selve's stress theory is now completely subsumed into the energy conservation theory. The present author proposes the mechanism of the hypophysis-suprarenal gland system as the direct control system of cellular energy metabolism of mitochondria in whole cells in the animal body and this is the immune system. The immune facility is the remodelling capacity conjugated with energy metabolism, which is the same as the cellular digestion system. Therefore, the hypophysis-suprarenal gland system (i.e., the direct control system of mitochondrial metabolism in whole cells) is the regu lating system of energy metabolism as well as the acceptor of the stimuli of energy and substances with mass, including bacteria. The cause of intractable human-specific immune diseases is brought about through improper levels of energy influencing organisms and subsequent intracellular infections of specific organs by pathogenic as well as nonpathogenic parasites-enterobacteria or viruses. The energies are gravity, cold drinks and food. atmospheric pressure, thermal and electromagnetic stimuli and sunlight. Most intracellular infections of cells are caused by parasitic enteric bacteria.

5. RESEARCH ON THE DISCLOSURE OF MAJOR CAUSES OF MITOCHONDRIAL MUTATION BY MEANS OF MOLECULAR BIOLOGY

As already mentioned, mitochondria are the most important organelles in the mammalian life system. Therefore, to disclose the cause of mitochondrial mutation and/or deterioration is today's urgent issue. It is well known that in mitochondrial diseases as well as alcoholism, cancer and infection by rickettsia and viruses, mitochondrial mutation, mitochondrial disorder, mitochondrial dysfunction and mitochondrial abnormalities or morphological disorders are observed in intractably infected cells. In today's clinical medicine, not only in mitochondrial diseases but in various immune diseases, mitochondrial mutations are commonly reported. The causes of these mutations are

considered to be the effects of free radicals of oxygen generated during the mitochondrial function of oxidative phosphorylation. However, the author is sceptical about this concept of mutation by free radicals of oxygen. About 40 years ago the theme of the present author's PhD thesis was "Disclosure of Major Causes of Mitochondrial Mutation by means of Molecular Biology" (published in Japanese). Some papers were published in English on this topic (Nishihara, 2008b and 2009a). Part I of the thesis aimed to reveal the mechanism of mitochondrial mutation, their deformities and deterioration in diseased cells. The author carried out the following model experiments using yeast *(Saccharomyces cerevisiae):* (1) Experiment for development of respiration-deficient strain using an inhibitor of protein synthesis in cytoplasm (cycloheximide) and of mitochondria (chloramphenicol) in culture; (2) Measurement of the activities of mitochondrial DNA and RNA synthesis *in vitro* during development of a respiration-deficient strain using an inhibitor of protein synthesis in culture. Part 2 concerned the interaction between nuclei and mitochondrial genes during the development of organelle mitochondria. To reveal the developmental mechanism of mitochondrial mutants the author carried out molecular biology experiments using wild strains of yeast and several respiration-deficient strains of different genotypes, in which he observed *in vitro* the synthesis of DNA polymerase and RNA polymerase of mitochondria. Part 3 dealt with mitochondrial genes: observation of the activities of yeast mitochondrial ATPase with the administration of cycloheximide cytoplasmic protein synthesis were measured.

From the experiments of Part 1 the following results were obtained: I, using cycloheximide, a respiration-deficient strain (i.e., petite mutant) could be obtained at a high rate. 2, using chloramphenicol, no marked development of a respiration-deficient strain was obtained. 3, using streptovalicin, no marked development of a respiration-deficient strain was obtained. 4, in yeast cultured in cycloheximide-containing medium decreased activities of mitochondrial DNA polymerase, and no activities of mitochondrial RNA polymerase, were observed. 5, in yeast cultured in chloramphenicol-containg medium, markedly increased activities of mitochondrial DNA as well as of mitochondrial RNA were observed.

From these results the author concluded that the petite (respiration-deficient) mutant can be developed by an inhibitor not of mitochondrial but of nucleic cytoplasmic protein synthesis, which would disturb mitochondrial DNA polymerase as well as RNA polymerase synthesis in the cytoplasm.

From the experiments of Part 2 (measurement of DNA/RNA synthesis) the following results on specific activities of wild-type strain and strains with mitochondrial mutation were obtained: I, DNA synthesis of epistatic mutant 5d was extremely high. 2, lowering of RNA synthesis was observed with 5d and segregational mutant 431. 3, extreme inhibition by the operation of chloramphenicol and cycloheximide in RNA synthesis was observed with mutant 431. It was inferred from these results that the developmental mechanism of mitochondrial mutation was a disturbance of mitochondrial DNA and RNA polymerase due to cycloheximide.

From the experiments of Part 3 the following results were obtained: I, inhibitor did not show any difference from that of the control. 2, the activities of yeast mitochondrial ATPase with the administration of chloramphenicol showed evident lowering. 3, the activities of yeast mitochondrial ATPase with the administration of ethidium bromide showed evident lowering. With the above results, an interpretation would be allowed that coupling factor F_1 (ATPase) is controlled by mitochondrial genes, and that its genetic information is translated by the protein synthesis system of mitochondria. By revealing the functions of mitochondrial genetic information, it is expected to obtain clues to determine whether the morphological transformation of mitochondria in diseased or cancerous cells is derived from the disturbance of synthesis of DNA polymerase and RNA polymerase of mitochondria, which are encoded in nuclear genes.

From these model experiments using yeast, one of the causes of respiration-deficient mutants was shown to be a disturbance of DNA polymerase as well as of RNA polymerase of mitochondria due to cycloheximide. After clinical studies to cure intractable immune diseases, instead of cycloheximide, tremendous numbers of intracellularly infected nonpathogenic enterobacteria or viruses would induce severe disturbance of protein synthesis in cytoplasm (Nishihara, 2009a). Hence, the author proposes that the major cause of intractable immune diseases and mental illness is a result of mitochondrial deterioration due to entangled complicated intracellular contamination by low virulence pathogenic as well as nonpathogenic common enteromicrobes such as viruses, mycoplasma, ricketia, chlamydia and/or bacteria in various organ cells and in neurons.

6. HUMAN-SPECIFIC INTRACTABLE IMMUNE DISEASES-THE HYPOTHESIS OF MITOCHONDRIAL DETERIORATION VIA INTRACELLULAR INFECTION OF ORGAN CELLS IN THE HUMAN BODY

Human-specific intractable immune diseases are generally accepted as autoimmune diseases in modern

medicine; i.e., self and nonself immunology, in which leukocytes recognize mistakenly as nonself and revolt against their own cells with their MHC (HLA). The causes of these immune diseases have not yet been discovered, even though they are commonplace in today's modern lifestyle. Confusion and disordered immunology that does not serve to cure maladies started from the concept of immune tolerance emerging from Medawar and Le Douarin's experiments. This is an issue of organ transplantation in animals and not an issue of immunity to infectious maladies. Therefore this is tissue immunity; i.e., animal leukocytes reacting against transplanted organ cells, not immunity reacting against bacteria or viruses. From Le Douarin's research on chick quail chimeric embryos, it was discovered that if quail neural crests of brain or wings are transplanted concomitantly with the thymus, chimeric chicks could grow up without rejection (Le Douarin, 1982). However, without concomitant quail thymus transplantation to the chick embryo, after the chimera grew up the transplanted quail organs would be rejected. The thymus is the most important haemopoietic organ in mammals and birds for generating characteristic T cells. If this mistaken concept of immunology becomes established, this new pseudoscience will exert a strong influence upon the treatment of immune diseases with mistaken therapeutic concepts. Therefore, this immunology has to be completely destroyed to return therapeutic medicine to its proper place for the treatment of intractable immune diseases.

The present author has confirmed from clinical research that upon cooling the gut by just 1°C from *37°C,* intracellular infection of leukocytes occurs via M cells in Peyer's patch, which develop into granulocytes. Granuloc}tes contaminated with numerous bacteria circulate in the whole body disseminating bacteria into various organ cells resulting in intracellular infection of these organs. The author hypothesizes that these intractable immune diseases are not autoimmune diseases but severe cases offonnally accepted opportunistic infections, which are caused by intracellular infection by self common nonpathogenic enteromicrobes (Nishihara, 2006).

Contaminated granulocytes from the pus of periodontitis, the sputa of lung diseases or the sedimentation of the urine of nephritis contain numerous moving bacteria, as can be observed highly magnified (x3000) in the light microscope. These intracellular infections deteriorate as well as mutate mitochondria, and result in functional disturbances of specialized organs, which appear to be immune diseases. The author hypothesizes that human-specific intractable immune diseases are severe cases of opportunistic infections or autotoxic diseases caused by intracellular infection of common nonpathogenic enterobacteria and/or enteroviruses as a result of lifestyle changes. The author also hypothesizes that by intracellular infection of common enterobacteria and/or enteroviruses mitochondrial deterioration and mutation in the cytoplasm takes place. Subsequently, the author verified the intracellular infections of common enterobacteria, observing the leukocytes as well as epithelial cells obtained from sedimentation of sputa of lung diseases or urine of nephritis using highly magnified (x3000) light microscopy or transmission electron ultramicroscopy (TEM).

With these hypotheses and understanding, the author established the mitochondrion-activating therapeutic method (MATM) to cure those diseases by means of prevention and recovery from intracellular infections in conjugation with nose breathing during sleep as well as warming the gut, recovering bone rest time by lying down, moderate eating and drinking with optimal mastication, treating periodontitis, optimal exposure to sunshine by sunbathing, and by administering suitable bifidus factors, effective antiviral agents as well as antibiotics. By these MATM remedies intracellularly infected microbes are controlled, mitochondrial mutation and/or deterioration are easily countered and the specific functions of specialized organ cells are restored completely. In most of the cases, the patients who had been diagnosed with intractable immune diseases in proper hospitals showed evident recovery by these curative methods. From the complete cures of intractable immune diseases, namely complete recovery of deteriorated mitochondria in diseased cells via these MATM remedies, the hypotheses are verified as diagnosis *ex juvantibus;* i.e., diagnosis based on the results of treatment (Nishihara, 2009a). If intracellular infection occurs in some organ, the function of the cells of the organ deteriorates because of the dysfunction of mitochondria caused by contaminating bacteria or viruses. This is an immune disease (Nishihara, 2004a). These intracellular parasites of specific cells hinder the energy metabolism of mitochondria, leading to deterioration of organ function and to intractable immune diseases. Immune diseases are a hindrance of cellular renewal (remodelling), which is conjugated with the energy metabolism of mitochondria. The causes of most immune diseases are a deterioration of the mitochondrial function by various energies as well as by intracel1uJarly-infected bacteria or viruses; i.e., parasites. Intracellular contamination of specially differentiated cells (e.g., nemons or hormonal glands) by parasitic microbes of the gut, regardless whether aerobic or anaerobic, disturbs the specialized function of mitochondria. This is the immune disease condition at the subcellular level

(Nishihara, 2004a). Cytoplasmic protein synthesis would be disturbed via intracellular infection instead of cycloheximide, which is an inhibitor of protein synthesis of eukaryotae (Nishihara, 2008b), consequently mitochondrial mutation as well as deterioration occur.

7. ESTABLISHMENTOFGENIJINECLINICALIJHMUNOLOGY, AS WELL AS THERAPEUTIC METHODS TO OVERCOME AGING BY MEANS OF CONTROLLING ENVIRONMENTAL ENERGYAND MITOCHONDRIAL ENERGY METABOLISM

To establish genuine clinical immunology, it is necessary to define what intractable immune diseases are. As already described, immune diseases are a cellular-level intracellular infection of tremendous numbers of nonpathogenic or low virulence enteromicrobes in various organs or tissue cells. Consequently, deterioration of mitochondria occur. As a result, specialized functions of the organ or tissue cells are disturbed. Actually, the specialized functions of cells in specific organ cells depend completely upon mitochondrial activities. Therefore, at the cellular level immune diseases are induced by mitochondrial deterioration. This occurs via the following six factors:

(1) Improper environmental energy
(2) Malnutrition, including oxygen
(3) Toxic substances
(4) Infection by pathogenic microbes and/or intracellular infection by nonpathogenic enteromicrobes
(5) Transplanted organs or tissues of animals
(6) Biological energy such as mind, belief, religion, spirituality, soul, fear, pain, loss and biological stresses.

These six items are very similar to the factors influencing mitochondria mentioned in Section 4, and aging factors of cells. All intractable diseases occur via intracellular infections and/or the other five items, which are brought about by dissemination of microbes from granulocytes induced by poor oral hygiene (i.e., periodontitis, breathing through the mouth, etc.).

Intractable diseases are largely placed into the following three categories. They are caused by intracellular infection of organ cells via common enteromicrobes. Thereafter, deterioration of the mitochondria occurs:

(I) Intractable immune diseases. Common immune diseases are intracellular infections of various tissues or organs.
(2) Mental illness. Intracellular infection in neurons in the brain. Mental illness is an organ (brain)-specific immune disease, in which intracellular infections occur in various parts of the cerebral neurons; e.g., in the visceral brain (i.e., the limbic system or in cerebral neurons). According to the site of the intracellularly infected neurons the symptom and name of the disease are decided.
(3) Carcinoma-complicated intracellular infection by multiple enteromicrobes in various tissues or organs. Carcinoma is a mode-specific immune disease in which intracellular infection of mu!tiple viruses and bacterial contamination occur, resulting in the deterioration of mitochondria, by which collapse of the negative feedback regulation system of cell division is brought about.

Sites of occurrence of common immune diseases and carcinoma are the same. In conventional medicine they are accepted as quite different kinds of diseases; however, they are the same immune diseases induced by a complicated intracellular infection by common enteromicrobes. The author has developed a method for anti-aging via activating mitochondria (MATM).

8. MULTIPLE FIJNCTIONS OF MITOCHONDRIA, ACTION OF STEROID HORMONES. ANDTHEAGING MECHANISM

The author has shown that the causes of human-specific intractable immune diseases are mitochondrial deterioration due to intracellular infection of common enteromicrobes. Through mitochondrial deterioration the life power of diseased cells markedly decreases, therefore remodelling of diseased cells is disturbed. Consequently aging of diseased cells take place. Therefore, aging of human life depends upon the mitochondrial condition, whether vital or fatigued or deteriorated. Steroid hormones are synthesized in mitochondria in suprarenal cortical glands and gonad glands and their targets are the mitochondria in al16 x 10^{13} cells (excepting matured erythrocytes) of mammals. Mineral- and glyco-corticosteroid hormones not only activate mitochondrial function haphazardly, but also some kinds of bacteria such as rickettsia, mycoplasma and chlamydia. Steroid hormones counter the inflammatory substances in the cytoplasm, which are produced by intracellularly contaminating microbes and induce itching, pain, redness and swelling. However, they cannot counter intracellularly contaminating bacteria and indeed promote their proliferation in the cytoplasm. Therefore, steroid hormones are simply effective at decreasing inflammatory symptoms but the maladies progress even though symptoms are subsiding. Moreover, by using steroid hormones for the symptomatic therapy of intractable immune diseases, aging of the diseased cells proceeds. Aging in the common sense is a deterioration of the constructive order of substance by environmental energy, according to elapsed time. Living organic substances constituting cells easily deteriorate through the action of cold or heat (thermal energy) in proportion to elapsed time. The systemic administration of synthetic steroid hormones for

symptomatic therapy for intractable immune diseases promotes the aging of vital activity of all cells in patients. The most important factor of the aging controlling mechanism depends upon the condition of the mitochondria. The causes of cellular aging are quite similar to the six factors causing mitochondrial deterioration in immune diseases as well as the six substances influencing mitochondria. The most important function of mitochondria is the energy metabolism of the TeA (Krebs) cycle, which generates the energy substance, ATP, with which haem proteins, cholesterol, steroid hormones, various kinds of cytokines, growth factors and signal transmission substances are synthesized in mitochondria.

Apoptosis is also controlled in mitochondria by a protease (caspase). The mechanism of apoptosis is still not precisely elucidated, but it is \vell known that mitochondria have a very important rôle in this phenomenon. The author proposes that mitochondria play a very important role in generating antiDNA antibodies, antiphospholipid antibodies, CRP and immunoglobulin E, which would be synthesized via mitochondria concomitant with genes as well as membranes of intracellularly infected microbes. The author considers that cell division-controlling substances on cell surface membranes ingrowth as well as injury regeneration are synthesized in mitochondria. which control cell division by a negative feedback regulation system in normal growth or recovery from injury. By intracellular infection of tremendous numbers of enteromicrobes deterioration of this negative feedback regulation system in mitochondria occurs; carcinoma develops from complicated intracellular infections with multiple microbes, which then grows rapidly with the possibility of metastasis.

As already mentioned, major causes of intractable immune diseases are intracellular infection by nonpathogenic common enteromicrobes, breathing through the mouth during sleep or prolonged speaking and/or ice intoxication, deteriorated oral hygiene or severe periodontitis. All intractable immune diseases occur from the same causes and mechanisms: via mitochondrial mutation and/or deterioration by inadequate absorption of environmental energy and by biomechanical energy evoked by habitual behaviour and by intracellularly disseminated common enteromicrobes through granulocytes. Therefore, all kinds of intractable immune diseases, including mental illness and carcinoma, can be prevented, treated, and cured by a proficient stomata-facial and neuro–cranial practitioner when the case is not too late, by means of mitochondria-activating immune disease therapeutic methods, which the author has developed. After all, by activating mitochondria with beneficent environmental energy as well as nutrition and respiration, aging can be overcome effectively by curing immune diseases completely.

REFERENCES

Caneva, K.L. *Robert Mayer and the Conservation of Energy.* Princeton: University Press (1993).

Le Douarin, N. *The Neural Crest*. Cambridge: University Press (1982).

Lehninger, L. *The Mitochondrion,* pp. 56-104. New York: Benjamin (1964).

Nishihara, K. and Tanaka. J. Successful inducement of hybrid type artificial bone marrow using bioceramics in various vertebrates. *Bioceramics* 9 (1996) 69-72.

Nishihara, K. Development of hybrid-type artificial immune organ by means of experimental evolutionary research method using bioceramics. In: *Tissue Engineering for Therapeutic Use 1* (eds Y. Ikada and Y. Yamaoka), pp. 39-50. Amsterdam (1997).

Nishihara, K. Evidence of biomechanics-evolutionary theory by using bioceramics. In: *Bioceramics* (eds H. Ohgushi, G.W. Hastings and T. Yoshikawa), vol. 12, pp. 253-256 (1999).

Nishihara, K. Evidence-based evolutionary research and development of the practical phylogenetics: verification of the gravity-corresponding evolutionary law by means of biomaterials. In: *Proc. 6th Meeting and Seminar on Ceramics, Cells and Tissues* (eds A. Ravaglioli and A. Krajewski), pp. 167-172. Faenza, Italy (2000).

Nishihara, K. Verification of the gravity action in the development of bone marrow hemopoiesis during terrestrialization. In: *Proc. 7th Meeting and Seminar on Ceramics, Cells and Tissues* (edsA. RavaglioliandA. Krajewski), pp. 277-288. Faenza, Italy (2001).

Nishihara, K. Differentiation into hemopoietic cells from mesenchymal cells in porous ceramic bone marrow chamber surface *in vivo* by means of hydrodynamics. In: *froc. 8th Meeting and Seminar on Ceramics, Cells and Tissues. Bioceramic surfaces* (eds A. Ravaglioli and A. Krajewski), pp. 157-162. Faenza, Italy (2003a).

Nishihara, K. Verification of use and disuse theory of Lamarck in vertebrates using biomaterials. *Biogenic Amines* 18 (2003b) 1-17.

Nishihara, K. Establishment of a new concept of the immune system, disclosure of causes, and development of the therapeutic system of immune diseases. *Biogenic Amines 18* (2004a) 79-93.

Nishihara, K. Research on the evolution and development of autonomic nervous system. *Biogenic Amines* 18 (2004b) 95-]06.

Nishihara, K. Trilateral research on neural system and biogenic amines: Disclosure of the major causes and mechanisms of human characteristic neurocerebromuscular (psychosomatic) disorders. *Biogenic Amines* 19 (2005) 197-208.

Nishihara, K. Disclosure of mechanisms of the mammalian life system and Selye's stress theory. *Biogenic Amines* 20 (2006) 171-184.

Nishihara, K. Disclosure of causes of human-specific intractable immune diseases-mitochondrial deterioration due to intracellular infections. *Biogenic Amines* 21 (2007a) 23-41.

Nishihara, K. Disclosure of causes of human-specific

intractable immune diseases by means of bio-energy resonance. Detection of mitochondrial deterioration due to intracellular infections using bi-digital O-ring test. In: *Proc. IIth Meeting and Seminar on Ceramics, Cells and Tissues* (edsA. Ravaglioli andA. Krajewski), pp. 278-285. Faenza.ltaly (2007b).

Nishihara, K. Development oftherapeutics for human-specific intractable immune diseases by means of bio-energy resonance-remedy of mitochondrial deterioration due to intracellular infections using bi-digital O-ring test. *Biogenic Amines* **22** (2008a) 75-84.

Nishihara, K. Disclosure of major causes of mitochondrial mutation by means of molecular biology. Part I. *Biogenic Amines* 22 (2008b) 99-1 14.

Nishihara, K. Human specific intractable immune diseases--the hypothesis and case presentation to disclose the causes and the cures. *Biogenic Amines* **23** (2009a) 55-74.

Nishihara, K. Disclosure of the causes of mental illness by means of diagnosis *ex-juvantihus* via bi-digital O-ring test. *Biogenic Amines* **23** (2009b) 253-273.

Schrbdinger. E. *What* is *Life?* Cambridge: University Press (1944).

Selye, H. Studies on adaptation. *Endocrinology* **21** (1937a) 169--188.

Selye, H. The significance of the adrenals for adaptation. *Science* **8S** (J 93 7b) 247--248.

Tache, Y. and Morley, J.E. *Neuropeptides and Stress.* New York: Springer (1989).